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Drinking Water Criteria Document for Toxaphene

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16. ABSTRACT

This document deals with the drinking water health effects of Toxaphene through the review of several studies. These studies include animal and humans. Physical and chemical properties are discussed. Carcinogenicity of the compound is reviewed and evaluated through various studies. Any existing guidelines and standards are presented.

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DRINKING WATER CRITERIA DOCUMENT FOR TOXAPHENE

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Office of Health and Environmental Assessment
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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1984; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
bw	Body weight
CNS	Central nervous system
DNA	Deoxyribonucleic acid
dw	Dry weight
DWEL	Drinking water equivalent level
GC	Gas chromatography
GI	Gastrointestinal
HA	Health advisory
i.p.	Intraperitoneal
i.v.	Intravenous
LD_{50}	Lethal dose to 50% of the recipients
LOAEL	Lowest-observed-adverse-effect level
MEL	Minimal effect level
MFO	Mixed function oxidase
NADPH	Nicotinamide adenine dinucleotide phosphate dehydrogenase
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
ppm	Parts per million
RfD	Reference dose
s.c.	Subcutaneous
TLC	Thin-layer chromatography
TLV	Threshold limit value
Toxicant A	Subfraction of toxaphene separated chromatographically
Toxicant A-1	A toxic component of Toxicant A
Toxicant A-2	A toxic component of Toxicant A
Toxicant Ac	A contaminant of Toxicant A
Toxicant B	Subfraction of toxaphene separated chromatographically
TWA	Time-weighted average
ww	Wet weight

I. SUMMARY

Toxaphene is a broad spectrum, chlorinated terpenoid pesticide introduced in the United States as a contact insecticide in 1948. Together with methoxychlor, toxaphene has been the chief replacement for DDT when use of DDT was banned. On May 25, 1977, the U.S. EPA issued a notice of rebuttable presumption against registration and continued registration of pesticidal products containing toxaphene. A further notice was issued in the Federal Register in 1982. Registration for use after December 31, 1983 for scabies treatment of beef cattle and sheep in vat dips and spray dips was still allowed under specified conditions. Toxaphene use to control sporadic infestations of army worms, cutworms and grasshoppers on cotton, corn and small grains was also permitted under specific conditions. Uses for control of mealybugs and pineapple gummosis moths on pineapples, and weevils in bananas are allowed only in the Virgin Islands and Puerto Rico. Sale and distribution of existing toxaphene stockpiles was allowed until December 31, 1986 with the use of toxaphene under limited conditions only. In addition to the previous uses, it may be applied to soybeans and peanuts for sicklepod control. Its sale and distribution for use as an insecticide in no-till corn and dry and southern peas was permitted until December 31, 1986. These administrative decisions were made as a result of the toxic effect of toxaphene on wildlife, aquatic organisms, nontarget organisms, and the potential oncogenicity of toxaphene in humans.

A variety of guidelines or standards have been published to protect against toxaphene in aquatic media. The national interim primary drinking water standard is 5 µg/ℓ. The FDA mandated level for bottled water is also 5 µg/ℓ. These standards are based upon organoleptic effects.

The water quality criterion for the protection of human health was originally set at 0.5 µg/ℓ for a 10^{-5} cancer risk level. This criterion was subsequently revised to 7.1 ng/ℓ for a risk of 10^{-5}. If consumption of aquatic organisms is considered alone, the corresponding risk level is 7.3 ng/ℓ. These criteria were based on the induction of hepatocellular tumors in mice.

Toxaphene is a mixture (average molecular weight 414) of at least 177 compounds, mostly chlorinated camphenes, which has been used as a broad spectrum contact pesticide (e.g., in soybean and cotton fields, and for ectoparasites). It is produced industrially by the reaction of camphene and chlorine in carbon tetrachloride solvent in the presence of ultraviolet light to produce a product of highly variable quality. Toxaphene can be provided as a 40% dust concentrate, as a liquid formulation containing 10% xylene, or as emulsifiable concentrates. It is extensively co-formulated with other pesticides (e.g., DDT, parathion, endrin).

The vapor pressure, density, solubility and other physical properties vary according to the quality of the product. Toxaphene is nonpolar and is soluble in nonpolar solvents; it is less soluble in polar solvents. It adsorbs to soils, and partitions readily into n-octanol. Because toxaphene is aliphatic, it is a poor absorber of ultraviolet light above wavelengths of 250 nm. Gas chromatography with electron capture detection or chemical ionization mass spectrometric detection are commonly used to measure toxaphene levels in biological samples. In a few cases, ^{1}H- and ^{13}C-nuclear magnetic resonance techniques have been used to assess the "fingerprints" of toxaphene fractions.

Toxaphene readily dehydrochlorinates on heating above 120°C, but the toxaphene subfractions Toxicants A and B appear to be stable on gas chromatography. Dehydrochlorination also occurs in the presence of alkali (used as an analytical method), of reduced hematin, or iron compounds. Reductive dechlorination occurs on ultraviolet light irradiation (230-290 nm) of toxaphene in organic solvents, preferentially at the C_2 and C_5 positions.

Most of the analytical techniques used to detect toxaphene metabolites have employed thin-layer chromatography as the end step. Toxaphene in environmental samples and tissues often does not have the chromatographic pattern of a toxaphene standard so that qualitative identification by gas chromatography occurs primarily through retention time; quantitation is generally accomplished by the method of alkaline dehydrochlorination, extraction and chromatography (Gomes method, detection limit 1 ng). Metabolites are generally isolated by thin-layer chromatography or liquid chromatography.

Though no quantitative data are available, the symptoms of toxicosis as well as the presence of residues and metabolites after exposure in animals, birds and humans indicate that toxaphene can be absorbed through the skin, the lungs, and the gut.

In White Leghorn chickens, accumulation of toxaphene residues occurs at doses of >12.5 mg toxaphene/kg bw/day. In ducks, brain residues were detected at concentrations >10 ppm in the feed. Extensive dechlorination of toxaphene by chlorinated organic residues occurs mostly in the fat. Doses less than those resulting in accumulation can be tolerated for months with little effect.

In mammals, storage of toxaphene in the fat has been reported in sheep, steers and dairy cows. Accumulation of toxaphene in sheep and steers occurred at administered concentrations between 10 and 100 mg toxaphene/kg feed/day. In contrast, dogs appear to store toxaphene derivatives in the brain. In male or female rats, fat levels of toxaphene were relative to concentrations >21 ppm in the diet. Studies using labeled toxaphene revealed rapid dechlorination and subsequent elimination of toxaphene, Toxicant A and Toxicant B at doses ≤19 mg toxaphene/kg bw administered by gavage to male rats using corn oil as a carrier. Toxaphene derivatives were measured in the blood, fat, liver and kidneys. When male rats were chronically dosed at 2.4 mg toxaphene/kg bw/day, plateau levels were found in the liver and brain after 1, 3 and 6 months. Transplacental transfer as well as blood-brain barrier transfer occurred in rats. The adrenal gland, carcass, cecum and abdominal fat of female rats contained toxaphene derivatives 3 days after an acute dosing with 2.6 mg toxaphene/kg bw. Extensive dechlorination and rapid elimination of metabolites also occurred in guinea pigs, hamsters, rabbits, mice and monkeys.

In rats, successful detoxification appeared to require NADPH and oxygen. Toxicants A and B undergo reductive dechlorination, dehydrochlorination, and vicinal chloride elimination. Involvement of the MFO system was suggested by the observation of Type I binding spectra with the hepatic cytochrome P-450 of rats, mice, sheep and rabbits, as well as the demonstrated enhanced toxicity to mice of toxaphene in the presence of piperonyl butoxide. There also appeared to be a detoxification pathway dependent upon glutathione. Toxicant B resisted metabolism more than Toxicant C. Glucuronide (major) and sulfate (minor) conjugates have been detected as metabolites. Toxicant C also produced one primary and four secondary alcohols, whereas reductive dechlorination predominated for Toxicant B. These data suggested that toxaphene components were not necessarily metabolized at the same loci in the microsomal system.

Elimination of products derived from toxaphene has been demonstrated to occur in the feces, urine and expired air of rats. The fecal route appears to be slightly more predominant than the urinary route. Milk and eggs may also contain residues. The species order of decreasing elimination efficiency for Toxicant B is as follows: monkey, rat, hamster > mouse, rabbit, guinea pig > chicken.

Acute oral exposure to toxaphene gave LD_{50} values ranging from 7.5-10 mg/kg bw in female monkeys to 75-500 mg/kg in rabbits. The LD_{50} values resulting from dermal application of toxaphene are generally higher than those observed after oral exposure; they range from ~250 mg/kg bw to ~4000 mg/kg bw with both values being reported for rabbits. The vehicle used with the pesticide may affect the toxic response. Specific components of technical grade toxaphene are more toxic than the complex mixture and, as reported for mice, LD_{50} values for several components varied from 2.5 to >100 mg/kg bw. Brain levels of toxaphene may be indicative of acute toxicity. In swine, >4 mg/kg ww (brain tissue) constituted a lethal level, and 2 mg/kg was associated with clinical signs of toxicity.

Subchronic oral doses of toxaphene in laboratory animals resulted in few clinical signs of poisoning. Lethality responses varied in mice fed 1280 ppm toxaphene in the diet for 6 weeks. No observable adverse effects were seen in rats following ingestion of feed containing 189 ppm toxaphene. At concentrations <189 ppm in the diet, subclinical lesions such as decreased bile production and questionable liver pathology were reported. In mice receiving 100 or 200 ppm toxaphene in the diet, humoral antibody production (IgG antibody formation) was suppressed; however, cell-mediated immune responses were not affected.

Inhaled toxaphene mist concentrations as high as 500 mg toxaphene/m^3 for 3 weeks caused no mortality in rats and rabbits; but at 12 mg toxaphene dust/m^3 for 3 months, mortality occurred in rats, dogs and guinea pigs.

Long-term exposures of animals to dietary levels of toxaphene are summarized in Chapter V. In a number of studies, no adverse effects were reported among the parameters monitored in each experiment. In several lifetime studies in rats, no effects were reported for dietary concentrations between 10 and 100 ppm toxaphene; other studies at 100 ppm toxaphene level reported liver pathology. The lowest dietary level producing liver damage in rats was 5 ppm for 2 years. In addition to liver pathology, a variety of adverse effects were reported from both _in vivo_ and _in vitro_ studies and included kidney pathology, decreased bile flow, decreased survival, inhibition of intestinal transport of glucose, inhibition of gluconeogenesis, inhibition of mitochondrial enzymes, inhibition of Na^+/K^+-ATPase brain and kidney activity, and inhibited Mg^{2+}-ATPase activity in mouse kidney, liver and brain.

Toxaphene induced the microsomal MFO system; treatment of animals _in vivo_ with a cytochrome P-450 system inhibitor increased toxaphene toxicity.

No effects on parental animals or offspring were noted in a three-generation study in which Sprague-Dawley rats were fed dietary levels of 25 or 100 ppm toxaphene. In a study using CD rats, maternal mortality was 31% at 35 mg toxaphene/kg bw. There was also a dose-related decrease in maternal weight gain and fetal weight (15, 25 or 35 mg/kg bw). No adverse effects were reported in the offspring of mixed-strain white rats dosed with 4 mg/kg bw during the entire pregnancy. In hamsters given the same dose, toxaphene was reported to be teratogenic but the anomaly was not identified.

More sensitive endpoints in rats were examined by several investigators. Toxaphene, at 12 mg/kg bw administered orally to pregnant rats, depressed cholinesterase activity in fetal cardiac neural structures and delayed cardiac neural differentiation. At 25 mg/kg bw, the only significant differences noted were decreases in alkaline phosphatase activity and total protein in the kidney. There was no effect at 12.5 mg/kg bw on the rat kidney. In two separate experiments there was little evidence of behavorial abnormalities in the offspring of toxaphene treated dams except for delayed righting ability (6 mg/kg and 50 μg/kg bw).

Toxaphene given to pregnant mice at 15 and 25 mg/kg bw had no adverse effect on parameters examined in offspring. However, a dose of 35 mg toxaphene/kg bw induced encephaloceles in five litters of 90 treated dams. In a 5- to 6-generation study where toxaphene (25 ppm) was given in the diet, little or no adverse effects were observed in mice. No effects were noted on the anatomical development of the fetal guinea pig from pregnant females given 15 mg/kg bw from days 21-35 of gestation.

Toxaphene appears to have a low teratogenic potential unless doses are large enough to induce maternal toxicity. In the studies reported here, the lowest dose producing an effect was 50 μg/kg bw (rats) given in the diet from day 5 of gestation through termination of the study. Effects included significant decreases in overall swimming ability and righting reflex of young pups when toxaphene was administered before postnatal day 16. In this study, however, the precise dose to the offspring is uncertain since they will receive toxaphene transplacentally, in the milk and in the feed.

Toxaphene is mutagenic in the Salmonella/microsomal reverse mutation assay using strains TA98 and TA100. Results of assays of toxaphene components for their mutagenic potential indicated that mutagenicity resided in the polar fraction. Mutagenicity was decreased by the addition of a liver microsome, S9, an active MFO (these results support the in vivo observation that inhibitors of cytochrome P-450 increased toxicity). The mutagenic activity of commercial preparations varies.

Studies of toxaphene carcinogenicity are reported in Chapter V. In an NCI carcinogenic study many doses were lowered because of the overt toxicity seen in rats and mice ingesting feed containing toxaphene. The concurrent numbers of control animals were low (10/sex/group) and historical controls were used in order to match the number of treated animals per group (50/sex/group). The range of doses given to the animals made it difficult to use time-weighted averages of doses as a basis on which to estimate risk for humans. It was concluded that under the conditions of the bioassay, toxaphene was carcinogenic in male and female $B6C3F_1$ mice, as evidenced by an increased incidence of hepatocellular carcinomas in a dose-related manner.

In a separate study using $B6C3F_1$ mice of both sexes, fed 7, 20 and 50 mg toxaphene/kg diet, the carcinogenicity of toxaphene was demonstrated at much lower doses. After 18 months of toxaphene ingestion and 6 months on a control diet, significantly increased incidences of hepatocellular carcinomas were detected in males. The response in females was less pronounced. Another study reported only one tumor, a subcutaneous neurosarcoma in rats exposed to 1000 ppm toxaphene in the diet.

Based on the positive carcinogenic evidence of toxaphene from the NCI mouse and rat studies and the other mouse study, it can be concluded that toxaphene is carcinogenic in at least two species of laboratory animals. Using the EPA criteria for classifying carcinogenicity data, the animal evidence would be considered "sufficient".

Toxaphene has caused convulsions and nausea in humans when exposure has occurred by ingestion, skin contact or inhalation. The acute oral LD_{50} was estimated from accidental poisonings to vary between 29 and 100 mg toxaphene/kg bw. Mixtures of toxaphene with DDT (2:1, by weight) are more acutely toxic than either component alone. The estimated hazardous dermal dose (applied to the skin) is ~660 mg/kg bw for liquid toxaphene. Allergic bronchopneumonia has been observed after acute inhalation of toxaphene sprays. Women have been shown to suffer a significant incidence of chromosomal aberrations in lymphocytes.

Dermal doses of 300 mg/day for 30 days, and inhaled doses of 0.4 mg/m^3 for 10 minutes on each of 15 days and 250 mg/m^3 for 30 minutes (~41 mg/day) on each of 13 days caused no atypical subjective or clinical effects.

Studies in the workplace are confounded because exposure to many chemicals had occurred in all the reported epidemiologic studies. There are no porphyrinogenic or sympathotonic effects mentioned in the available literature.

Human data are inadequate to assess the carcinogenic potential for toxaphene.

Animal studies were used to derive the 1- and 10-day HAs as a result of the lack of human data. The recommended 1-day health advisory (HA) for toxaphene in drinking water is 0.5 mg/ℓ for a 10 kg child. A NOAEL of 5 mg/kg bw based upon the onset of convulsions in dogs receiving a single dose of toxaphene was used to derive this HA. The recommended 10-day HA for toxaphene in drinking water is 0.04 mg/ℓ for a 10 kg child. A LOAEL of 4 mg/kg bw based upon minimal kidney and liver pathology in dogs was used to derive this HA.

There are no acceptable studies in the available literature for the derivation of a longer-term HA or a lifetime DWEL.

The recommended criterion for chronic exposure to toxaphene in drinking water, not considering intake from other sources, is 3.1, 0.31 and 0.031 µg/ℓ to maintain individual cancer risk less than 10^{-4}, 10^{-5} and 10^{-6}, respectively. This criterion is based upon an increase in hepatocellular tumors in male mice fed 7, 20 and 50 ppm toxaphene in the diet. The sufficient level of carcinogenic evidence in experimental animals, together with the inadequate level of human evidence meets the IARC Group 2B criteria for weight of evidence that a chemical is likely to be a human carcinogen. The assignment of a Group 2B designation by IARC means that the chemical is probably carcinogenic in humans. Applying the criteria described in the U.S. EPA's guidelines for assessment of carcinogen risk, toxaphene may be classified as Group B2: probable human carcinogen, meaning there is inadequate evidence from human studies and sufficient evidence from animal studies.

II. PHYSICAL AND CHEMICAL PROPERTIES

Toxaphene is a broad spectrum, chlorinated terpenoid pesticide introduced as a contact insecticide into the United States in 1948 (Brooks, 1974). Together with methoxychlor, it was and has been the chief replacement for DDT after DDT was banned. On May 25, 1977, the U.S. EPA issued a notice of rebuttable presumption against registration and continued registration of pesticidal products containing toxaphene (Federal Register, 1977). A further notice was issued in the Federal Register (1982). Registration for use after December 31, 1983 for scabies treatment of beef cattle and sheep in vat dips and spray dips was still permitted under specified conditions. Toxaphene use to control sporadic infestations of army worms, cutworms and grasshoppers on cotton, corn and small grains was also permitted under certain restrictions (Federal Register, 1982). The use of toxaphene to control mealybugs, gummosis moths on pineapples, and weevils in bananas is allowed only in the Virgin Islands and Puerto Rico. Depletion of existing toxaphene stockpiles was allowed until December 31, 1986 under limited conditions only. In addition to the previous uses, it may be applied to soybeans and peanuts for sicklepod control. Its sale and distribution for use as an insecticide in no-till corn and dry and southern peas was permitted until December 31, 1986 (Federal Register, 1982). These administrative decisions were made as a result of the toxic effect of toxaphene on wildlife, aquatic organisms, nontarget organisms, and potential oncogenicity of toxaphene in humans.

Toxaphene can be produced industrially by the reaction between technical grade camphene and chlorine (in carbon tetrachloride solvent) in the presence of ultraviolet light (mercury arc radiation) for 15-30 hours until the final product has a chlorine content between 67 and 69% (Sittig, 1977). The chemical quality of the final product is highly variable (IUPAC, 1979).

The many synonyms of toxaphene are provided in Table II-1. Toxaphene is known as chlorophene or polychlorocamphene in the Russian literature (Melnikov, 1971). Synonyms, chemical names and indexing terms for the major toxic components of toxaphene are given in Table II-2. The most toxic ingredients to mammals in technical toxaphene are Toxicant A and Toxicant B (Table II-2), each consisting of ~2-6% of the mixture (Metcalf, 1981; Khalifa et al., 1974). Toxicant A consists of three toxic components, Toxicant A-1 (CAS No. 58002-18-9) and Toxicant A-2 (CAS No. 58002-19-0), and a contaminant, Toxicant Ac (CAS No. 66860-80-8) (Chandurkar et al., 1978). Synonyms, chemical names and indexing terms for the major metabolites and degradation products of toxaphene are provided in Chapter III.

Since the technical grade product is produced by free radical reactions initiated by ultraviolet light, toxaphene is a complex mixture of polychlorinated camphenes and bornanes with an average empirical formula of $C_{10}H_{10}Cl_8$, and an average molecular weight of 414. In fact, over 177 incompletely characterized components have been separated (Holmstead et al., 1974). Technical grade toxaphene is an amber, waxy solid with a mild terpene odor, a softening range of 70-95°C, a vapor pressure of 0.17-0.40 mm Hg at 25°C, and a density of 1.66 g/mℓ at 27°C (Brooks, 1974). The solubility in water has been variously reported as ~3 mg/ℓ (Brooks, 1974) and 0.5 mg/ℓ at 25°C (Paris et al., 1977). Differing values for vapor pressure, solubilities and other physical constants are to be expected since

TABLE II-1

Nomenclature, Indexing Terms and Synonyms Currently Used for Toxaphene[a]

CAS RN 8001-35-2[b]

Empirical Formula: $C_{10}H_{10}Cl_8$

Toxaphene (DOT)[c] (8CI & 9CI)[d,e]
Agricide maggot killer (f)
Alltex
Alltox
Camphechlor
Camphochlor
Chem-Phene
Chlorinated camphene
Chlorinated camphene, tech
Chlor chem t-590
Compound 3956
Estonox
ENT 9,735
Fasco-Terpene
Geniphene
Gy-Phene
Hercules 3956
Kamfochlor
M 5055
Melipox
Motox
NCI-C 00259
Octachlorocamphene
Penphene
Phenacide
Phentox
Polychlorcamphene
Polychlorocamphene
PCHK
Strobane T
Synthetic 3956
Technical chlorinated camphene (67-69%) chlorine)
Toxadust
Toxafeen (Dutch)
Toxakil
Toxaphen (German)

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[a] Source: SANSS data base, June 1983

[b] Chemical Abstracts Service Registry Number

[c] Department of Transportation

[d] Eighth Collective Index, Chemical Abstracts

[e] Ninth Collection Index, Chemical Abstracts

TABLE II-2

Identified Toxic Components of Toxaphene

CAS RN MF	Chemical Abstracts Name (9CI)	Synonyms	Reference
51775-36-1 $C_{10}H_{11}Cl_7$	Bicyclo[2.2.1]heptane,2,2,5,6-tetrachloro-1,7,7,-tris(chloromethyl)-,(5-endo,6-exo)-	2,2,5-endo,6-exo,8,9,10-heptachlorobornane; Toxaphene toxicant B	Clark and Matsumura, 1979; Saleh and Casida, 1978; Saleh et al., 1979; Chandurkar and Matsumura, 1979a,b
52819-39-3 $C_{10}H_9Cl_9$	Bicyclo[2.2.1]heptane,2,3,3,5,6-pentachloro-7,7-bis(chloromethyl)-1-(dichloromethyl)-(2-endo,5-exo,6-exo)-	Toxaphene toxicant C*; 2-endo,3,3,5,6-exo,8,9,10,10-nonachlorobornane	Chandurkar and Matsumura, 1979a,b
57028-55-4 $C_{10}H_{10}Cl_8$	Bicyclo[2.2.1]heptane,2,2,5,6-tetrachloro-1,7-bis(chloromethyl)-7-(dichloromethyl)-	Toxic fraction A; 2,2,5-endo,6-exo,8,9,9,10-octachlorobornane	Clark and Matsumura, 1979
57981-30-3 $C_{10}H_{11}Cl_7$	Bicyclo[2.2.1]heptane,2,5,6-trichloro-3,3-bis(chloromethyl)-2-(dichloromethyl)-(exo,exo,exo)-	2,5,6-exo,8,8,9,10-heptachlorodihydrocamphene	Swanson et al., 1978
58002-18-9 $C_{10}H_{10}Cl_8$	Bicyclo[2.2.1]heptane,2,2,5,6-tetrachloro-1,7-bis(chloromethyl-7-(dichloromethyl)-(5-endo,6-exo,7-anti)-	2,2,5-endo,6-exo,8,8,9,10-octachlorobornane; Toxaphene toxicant A-1	Pollock and Kilgore, 1980
58002-19-0 $C_{10}H_{10}Cl_8$	Bicyclo[2.2.1]heptane,2,2,5,6-tetrachloro-1,7-bis(chloromethyl-7-(dichloromethyl)-(5-endo,6-exo,7-syn)-	2,2,5-endo,6-exo,8,9,9,10-octachlorobornane; Toxaphene toxicant A-2	Pollock and Kilgore, 1980
66860-80-8 $C_{10}H_9Cl_9$	Bicyclo[2.2.1]heptane,2,3,5,6-tetrachloro-7-(chloromethyl)-1-7-bis(dichloromethyl)-(2-endo,3-exo,5-endo,6-exo,7-syn)-	Toxaphene toxicant Ac	Chandurkar et al., 1978
70940-13-5 $C_{10}H_9Cl_9$	Bicyclo[2.2.1]heptane,2,3,3,5,6-pentachloro-1,7-bis(chloromethyl)-7-(dichloromethyl)-	Toxaphene toxicant C*; 2,3,3-endo,5,6-exo,8,9 10,10-nonachlorobornane	Clark and Matsumura, 1979

*Proposed structures have not been confirmed

CAS RN = Chemical Abstracts Service Registry Number; MF - Molecular Formula; 9CI - Ninth Collective Index, Chemical Abstracts toxaphene is a complex mixture and the quality of the commercial product is so variable. Thus, the lower chlorinated components will be more water soluble, and more volatile than those with a higher chlorine content. Odor detectibility by one individual of toxaphene dissolved in water has been found to be 0.14 mg/ℓ at 60°C (Sigworth, 1965).

Seiber (1982) noted the occurrence of differential volatilization. Toxaphene is miscible in acetone, benzene, carbon tetrachloride, ethylene dichloride, toluene and xylene; it is soluble (g/100 mℓ at 27°C) in turpentine (350-400), kerosene (>280), and fuel oil (250-275) (Brooks, 1974). Solubilities in alcohols such as isopropanol (15-18) and 95% ethanol (10-13) are generally lower. The heat of fusion is 0.39 cal/g; the specific heat is 0.258 cal/g/°C at 41°C; the viscosity is 1.4 poise at 100°C (Brooks, 1974). The octanol/water coefficient was variously found to be ~3300 (Paris et al., 1977) and 825 (Sanborn et al., 1976). The diffusion coefficient of toxaphene in water at 15°C has been estimated to be 5.6×10^{-6} cm^2 sec^{-1} (Veith and Lee, 1971). The K_{oc} value at 25°C is 98,600 (McDowell et al., 1981). The physical properties of these products are provided in Table II-3.

Toxaphene dehydochlorinates when heated above 120°C, or exposed to ultraviolet light (mercury arc) or intense sunlight (Brooks, 1974).

Toxaphene (20 mg of a 90% toxaphene/10% xylene liquid) when placed in a sealed tube for 15 minutes will produce different IR spectra at 250°C; volatilization occurs above 125°C (Kennedy et al., 1978). When heated in an open crucible in a muffle furnace for 45 minutes, the toxaphene/xylene

TABLE II-3

Physical Properties of the Degradation Products of Toxicant B[a]

CAS RN	Purity (%)	mp (°C)	Retention Time[b] (minutes)	^{63}Ni-EC Response Relative to Mirex[c]	Molecular Formula	Diagnostic CI/MS Peaks
57981-29-0	99	155-156	14.00	23	$C_{10}H_{12}Cl_6$	M-Cl, M-HCl, M-Cl-HCl
64618-63-9	99	107-108	11.15	23	$C_{10}H_{10}Cl_6$	M+1, M-Cl, M-HCl M-Cl-HCl
65620-64-6	90	146-148	13.23	13	$C_{10}H_{12}Cl_6$	M-Cl, M-HCl, M-Cl-HCl
66157-70-8	99	100-101	8.45	11	$C_{10}H_{11}Cl_5$	M+1, M-Cl, M-HCl

[a] Source: Saleh and Casida, 1978

[b] The retention time of Mirex on an open tubular column at 200°C was 45.50 minutes; the retention time of toxicant B was 20.41 minutes, and its relative EC-response was 51.

[c] EC = Electron Capture; Mirex response is taken to be 100.

CAS RN = Chemical Abstracts Service Registry Number mixture showed a weight loss of 94.2% at 400°C and 99.9% at 1000°C. Noticeably different gas chromatograms were noted at 300°C for the open crucible experiments. At 400°C, the major gaseous products were tentatively identified as hydrochloric acid, chlorine, phosgene, carbon tetrachloride, chlorinated aliphatics containing two carbons, carbon dioxide, carbon monoxide and water. In catalytic hydrodechlorination, the geminal chlorines are more readily attacked than the other chlorines (Kranich et al., 1978). Thus, <2% of toxaphene degradation products containing more than five chlorines remained after 19 hours of reaction (at 100°C, 50 bar pressure); a 10 g toxaphene in ethanol per gram Ni on Kieselguhr was used as a catalyst. Toxicants A and B appear to be stable under the conditions of gas chromatography (Khalifa et al., 1974).

Dehydrochlorination reactions are accelerated in the presence of alkalis and iron compounds (Brooks, 1974), that is, when toxaphene is refluxed in alcoholic potassium hydroxide (Archer and Crosby, 1966; Gomes, 1977; Crist et al., 1980), or when toxaphene is in the presence of hematin/sodium thiosulfate (Khalifa et al., 1976).

The colorimetric technique formerly used depended upon the reaction of toxaphene with diphenylamine and zinc chloride at 205°C to give a chromophore with a λ_{max} of 640 nm (Graupner and Dunn, 1960).

Studies on Toxicant B were reported by Saleh and Casida (1978). Toxicant B undergoes reductive dechlorination at the germinal dichloro group to yield 2-endo,5-endo,6-exo,8,9,10-hexachlorobornane and 2-exo,5-endo,6-exo,8,9,10-hexachlorobornane in many systems.

Production and Usage

Toxaphene production in the United States was 18.4 Mkg in 1978, and accounted for more than 13% of total cyclic pesticide production. Three manufacturers who reported their production or sales figures for that year were Tenneco Chemicals, Inc., Hercules, Inc. and Vertac, Inc. Vicksburg Plant (USITC, 1979). The United States toxaphene consumption for the years 1978, 1979 and 1980 was 15.5, 13.5 and 13 Mkg, respectively (FAO, 1982). In 1980, ~1 Mkg was applied to soybean acreage. This 1980 usage represented 23.8% of the total insecticides applied to this crop (Eichers and Serletis, 1982). During fiscal year 1980 toxaphene was also used by the U.S. Forest Service for tick and lice control on 3030 cattle. A total of 1607 acres (650 ha) were involved in this process (USDA, 1981).

Primary utilization occurs in agricultural crop applications, especially cotton (IUPAC, 1979). It is also extensively used for the control of exoparasites on livestock. The application rates vary from 1.2-9.6 kg/m^3 water (IUPAC, 1979). The cumulative world usage of toxaphene from 1946 to 1974 was ~450,000 tons (IUPAC, 1979).

Technical grade toxaphene can be supplied as a 40% dust concentrate and as a viscous liquid containing 10% xylene (Brooks, 1974), with the liquid being vulnerable to degradation by metals, such as iron and aluminum. Emulsifiable concentrates containing 4, 6 or 8 lb. of toxaphene/U.S. gallon (0.48, 0.72 or 0.96 kg/ℓ) are formulated usually with xylene, 0.5% epichlorohydrin and an appropriate emulsifier (if water is the carrier). Wettable powders (40% toxaphene content), dusts (5-20%) and granules (5 and 20%) are also used (Brooks, 1974; IUPAC, 1979). Toxaphene has also been coformulated with other pesticides [e.g. DDT, Maneb, Zineb, dioxathion, diazinon, malathion, methylparathion, endrin, endosulfan, isobornyl thiocyanoacetate plus related toxaphene terpenoids (Thanite), pyrethrins, and piperonyl butoxide] (Brooks, 1974). A selected list of recently used coformulations is provided in Table II-4. In addition to such uses toxaphene has been used as a protective agent to reduce phytotoxic effects of such other pesticides as phosphorothiolates (Aller and Hansen, 1981), to provide broad spectrum pesticidal properties in combination with such herbicides as benazolin (Anonymous, 1982), and to produce more potent insecticidal mixtures, i.e. with Amitraz (Kerry and Weighton, 1979).

Spectroscopic Properties

Toxaphene components are not aromatic and, therefore, would be expected to absorb light at wavelengths below 250 nm.

Infrared analysis is still used for qualitative identification. There are prominent absorption bands in the 6-8 μm region and the specified absorbence at 7.2 μm of 0.0177 (maximum) is utilized (Brooks, 1974). The infrared spectrum of toxaphene was reported by Archer and Crosby (1966). Since toxaphene is a complex mixture, mass spectroscopy (MS) of the mixture is not diagnostic. However, upon gas chromatography/mass spectroscopy (GC/MS), the presence of the most toxic components can be found if adequate resolution can be obtained on the gas chromatographic column. The electron impact and chemical ionization mass spectra of Toxicant A and B (see Table II-2) were recorded in Holmstead et al. (1974). Because the compounds are

TABLE II-4

Chemical Information Regarding Toxaphene Mixtures

CAS RN	Molecular Formula	Chemical Names	Synonyms
8073-68-5	$C_4H_8N_2O_2S$·Unknown	Ethanimidothioic acid,N-((aminocarbonyl)oxy)-, methyl ester, mixt. with toxaphene (9CI)	
8077-22-3	$C_{14}H_9Cl_5$·$C_8H_{10}NO_5PS$ Unknown	Phosphorothioic acid,O,O-dimethyl-O-(4-nitrophenyl)ester, mixt. with toxaphene and 1,1'-(2,2,2-trichloroethylidene)bis(4-chlorobenzene) (9CI)	Hexatox super; S 137b; Torbidan; DDT-toxaphene-methyl parathion mixture
37271-99-1	$C_8H_{10}NO_5PS$·Unknown	Phosphorothioic acid,O,O-dimethyl-O-(4-nitrophenyl)ester, mixt. with toxaphene (9CI)	
37272-06-3	$C_{14}H_9Cl_5$·Unknown	Benzene,1,1'-(2,2,2-trichloroethylidene)bis(4-chloro-, mixt. with toxaphene (9CI)	Toxaphene-DDT mixt.; DDT-toxaphene mixture; DDTox.; Polidofen; Hellotox; DDT-chlorinated terpene mixture
39295-90-4	$C_{10}H_{13}ClN_2$·Unknown	Methanimidamide,N'-(4-chloro-2-methylphenyl)-N,N-dimethyl- mixt. with toxaphene (9CI)	Toxaphene-galecron mixture
39295-93-7	$C_{13}H_{10}BrCl_2O_2PS$·Unknown	Phosphonothioic acid,phenyl-,O-(4-bromo-2-5-dichlorophenyl)O-methyl ester, mixt. with toxaphene (9CI)	Toxaphene phosvel mixture
39295-94-8	$C_{14}H_{14}NO_4PS$·Unknown	Phosphonothioic acid,phenyl-O-ethyl-O-(4-nitrophenyl)ester, mixt. with toxaphene ((9CI)	Toxaphene-EPN mixture
39400-89-0	$C_{15}H_{10}Cl_4$·Unknown	Benzene,1,1'-(2,2-dichlorocyclopropylidene)bis(4-chloro-, mixt. with toxaphene (9CI)	
39474-75 4	$C_{12}H_{11}NO_2$ ·$C_5H_{12}NO_3PS_2$·Unknown	Phosphorodithioic acid,O,O-dimethyl-S-(2-(methylamino)-2-oxoethyl)ester, mixt. with 1-naphthalenyl methylcarbamate and toxaphene (9CI)	
50641-56-0	$C_{10}H_{14}NO_5PS$·Unknown	Phosphorothioic acid,O,O-diethyl-O-(4-nitrophenyl)ester, mixt. with toxaphene (9CI)	Parathion-toxaphene mixture
55267-24-8	$C_{16}H_{15}Cl_3O_2$·Unknown	Benzene,1,1'-(2,2,2-trichloroethylidene)bis(4-methoxy-, mixt. with toxaphene (9CI)	
58386-60-0	$C_{10}H_{19}O_6PS_2$·Unknown	Butanedioic acid,((dimethoxyphosphinothioyl)thio)-,diethyl ester, mixt. with toxaphene (9CI)	Malathion-toxaphene mixture

TABLE II-4 (cont.)

CAS RN	Molecular Formula	Chemical Names	Synonyms
58703-96-1	$C_{14}H_9Cl_5 \cdot C_{10}H_{14}NO_5PS$·Unknown	Phosphorothioic acid,0,0-diethyl-0-(4-nitrophenyl)ester, mixt. with toxaphene and 1,1'-(2,2,2-trichloroethylidene)bis(4-chlorobenzene) (9CI)	Hexatox
58934-38-6	$C_6H_6Cl_6$·Unknown	Cyclohexane,1,2,3,4,5,6-hexachloro-,(1 alpha, 2 alpha, 3 beta, 4 alpha, 5 alpha, 6 beta)-, mixt. with toxaphene (9CI)	TXL
64034-61-3	CH_4N_2O·Unknown	Urea, mixt. with toxaphene (9CI)	
67872-25-7	$C_9H_{11}Cl_3NO_3PS \cdot C_8H_{10}NO_5PS$·Unknown	Phosphorothioic acid, 0,0-diethyl-0-(3,5,6-trichloro-2-pyridinyl)ester, mixt. with 0,0 dimethyl-0-(4-nitrophenyl)phosphorothioate and toxaphene (9CI)	
70020-45 4	$C_4H_7Br_2Cl_2O_4P$·Unknown	Phosphoric acid,1,2-dibromo-2,2-dichloroethyl dimethyl ester, mixt. with toxaphene (9CI)	Toxaphene-naled mixture
70162-18-4	$C_{19}H_{23}N_3$·Unknown	Methanimidamide, N'-(2,4-dimethylphenyl)-N-(((2,4-dimethylphenyl)imino)methyl)-N-methyl-, mixt. with toxaphene (9CI)	
73755-37-0	$C_{10}H_{13}ClN_2 \cdot C_8H_{10}NO_5PS$·Unknown	Phosphorothioic acid, 0,0-dimethyl-0-(4-nitrophenyl)ester, mixt. with N'-(4-chloro-2-methylphenyl)-N,N-dimethylmethanimidamide and toxaphene (9CI)	
77518-58-2	$C_{11}H_{14}Cl_3O_3PS$·Unknown	Phosphorothioic acid, 0-ethyl-S-propyl-0-(2,4,6-trichlorophenyl)ester, mixt. with toxaphene (9CI)	
77518-59-3	$C_{11}H_{15}BrClO_3PS$·Unknown	Phosphorothioic acid, 0-(4-bromo-2-chlorophenyl)-0-ethyl-S-propyl ester, mixt. with toxaphene (9CI)	
77837-51-5	$C_{25}H_{22}ClNO_3$·Unknown	Benzeneacetic acid,4-chloro-alpha-(1-methylethyl)-,cyano(3-phenoxyphenyl)methyl ester, mixt. with toxaphene (9CI)	
77837-52-6	$C_{21}H_{20}Cl_2O_3$·Unknown	Cyclopropanecarboxylic acid,3-(2,2-dichloroethenyl)-2,2-dimethyl-,(3-phenoxyphenyl)methyl ester, mixt. with toxaphene (9CI)	

CAS RN = Chemical Abstracts Service Registry Number; 9CI = Ninth Collective Index, Chemical Abstracts alicyclic, the mass electron impact spectra have extremely weak or nonexistent parent ion peaks, the major intense peaks being in the low m/e range. Thus, chemical ionization MS after GC is more useful than electron impact MS (Holmstead et al., 1974).

Although capillary GC showed Toxicant A was one peak, 90 and 270 MHz 'H-NMR spectroscopy revealed it to consist of two major components (Matsumura et al., 1975). The structure of Toxicant Ac was deduced primarily through its 'H-NMR spectra (Chandurkar et al., 1978), since its gas chromatographic properties were similar to those of components A-1 and A-2.

Saleh and Casida (1978) recorded the chemical ionization/mass spectra (CI/MS) of the major in vitro and in vivo degradation products of Toxicant B (see Chapter III). The chemical ionization/mass spectrometric (CI-MS) data can be found in Table III-7.

The 'H-NMR spectra of Toxicant C and its major metabolite (CR 70459-31-3) (Table III-7) was reported by Chandurkar and Matsumura (1979b).

Analysis

The major recent analytical methods for toxaphene in a variety of matrices are summarized in Table II-5. Most of the methods employ extraction from the suitably prepared substrate, a Florisil clean-up step, and electron capture/gas chromatography (EC/GC), or GC/MS. In the case of tissue analysis, a silica gel clean-up step is also often utilized before EC/GC or GC/MS (Haseltine et al., 1980). A nonpolar phase like OV-101 is generally utilized for gas chromatography.

TABLE II-5

Examples of Analytical Methods for Toxaphene

Substrate	Method	Concentration Added	Recoveries	Reference
Primary sludge (20 g)	Extraction; concentration; Florisil column cleanup; EC/GC	50 µg	92±27% 84±2% 91±7%	Rodriquez et al., 1982
		15 µg	89±20% 89±19% 93±22%	
Duck tissues (brain, liver, blood, eggs and carcasses)	Extraction; Florisil column cleanup; silicic acid separation; EC/GC (Gel permeation chromatography for blood and young carcasses)	10-20 ppm	113% (average)	Haseltine et al., 1980
Soil (1 g)	Florisil extraction and cleanup; Dehydrochlorination with KOH; GC	1.0 µg 0.5 µg 0.1 µg	84.9% 71.4% 71.1%	Crist et al., 1980
Bovine defibrinated whole blood circulated through a perfusion apparatus (2 mℓ)	I. Agitation with H_2O and hexane; centrifuge; hexane extraction; GC	20 µg/mℓ	73.4%	Maiorino et al., 1980
	II. Agitation with 88% formic acid and hexane; solvent phase shaken with K_2CO_3; hexane extraction; GC	20 µg/mℓ	71.7%	
	III. Agitation with 88% formic acid; Florisil extraction and cleanup; GC	20 µg/mℓ 0.3-15 µg/mℓ	103.4% 96.8-102.1% (range)	
Milk (20 mℓ)	5% Diethyl ether-hexane elution; GPC solvent isolation; acid oxidation; hexane extraction; Florisil column cleanup; EC/GC	0.5-5.0 ppm	78-88% (range)	Boshoff and Pretorius, 1979
Butter (1 g fat)	5% Diethyl ether-hexane elution; GPC solvent isolation; acid oxidation; hexane extraction; Florisil column cleanup; EC/GC	0.5-5.0 ppm	79-86% (range)	
Meat (10 g)	5% Diethyl ether-hexane elution; GPC solvent isolation; acid oxidation; hexane extraction; Florisil column cleanup; EC/GC	0.5-5.0 ppm	76-79% (range)	

Since toxaphene is a complex mixture, the chromatographic pattern of standards may not match that of the residual toxaphene remaining in environmental samples, since differential volatilization, sorption, solubilization or microbial degradation may have occurred that changed the diagnostic pattern. However, certain components of toxaphene are more toxic than others; these components can be selectively quantitated by the methods given in Table II-5. GC/MS techniques are invariably the ultimate forms of analysis after a EC/GC screening step, with chemical ionization MS being preferred since electron impact MS produces no or very weak molecular ions.

Some of the older methods for detecting toxaphene such as total organic chloride, colorimetric methods, or bioassays using insects, were generally nonspecific. Since toxaphene is an extremely complex mixture, and not very heat stable, the question of thermal degradation on GC columns remains a vexing one. Although there are three major chromatographic peaks (Gomes, 1977), the use of a flash heater filled with various reagents in the injection port produced substantial changes in the chromatogram (Minyard and Jackson, 1965). Treatment of toxaphene residues with alcoholic potassium hydroxide followed by column chromatography (detection to 1 ng) causes dehydrohalogenation with the appearance of one large single peak (Archer and Crosby, 1966; Gomes, 1977; Crist et al., 1980). Recoveries from soil are claimed to average between 71 and 85% at levels from 0.1-1 ppm toxaphene in soil, although the method did not appear to be useful for rat adipose tissue (Crist et al., 1980).

Reviews by Seiber (1982) and Pollock and Kilgore (1978a) highlight the difficulties of separating toxaphene from other chlorinated hydrocarbons, and pesticides.

Analysis for toxaphene metabolites has involved extraction techniques followed by thin-layer chromatography (TLC) (Chandurkar and Matsumura, 1979a,b). Pollock and Kilgore (1980) used TLC to circumvent artifacts caused by thermal degradation on GC columns.

Summary

Toxaphene is a mixture (average molecular weight 414) of at least 177 compounds, mostly chlorinated camphenes, which has been used as a broad spectrum contact pesticide (e.g., in soybean and cotton fields, and for ectoparasites). It is produced industrially by the reaction of camphene and chlorine (in carbon tetrachloride solvent) in the presence of ultraviolet light to produce a product of highly variable quality. Toxaphene can exist as a 40% dust concentrate, a 9:1 liquid mixture containing 10% xylene or as emulsifiable concentrates. It is extensively coformulated with other pesticides (e.g., DDT, parathion, endrin).

The vapor pressure, density, solubility and other physical properties vary according to the quality of the product. Toxaphene is nonpolar and so is miscible in nonpolar solvents, but less soluble in polar solvents. It adsorbs avidly to soils and partitions readily into n-octanol. Toxaphene, being aliphatic, is a poor absorber of ultraviolet light above wavelengths of 250 nm. Gas chromatography with electron capture or chemical ionization mass spectrometric detection are the most commonly used analytical methods. ^{1}H- and ^{13}C-nuclear magnetic resonance techniques may be used to assess the "fingerprints" of toxaphene fractions.

Toxaphene readily dehydrochlorinates when heated above 120°C, but Toxicants A and B appeared stable in gas chromatography. Dehydrochlorination also occurs in the presence of alkali (used as an analytical method), of reduced hematin, and of iron compounds. Reductive dechlorination occurs on exposure to ultraviolet light in the radiation range (230-290 nm) of toxaphene in organic solvents, preferentially at the C_2 and C_5 positions.

Most of the analytical techniques to detect toxaphene metabolites have utilized thin-layer chromatography as the end step. Toxaphene in environmental samples and tissues often does not have the chromatographic pattern of a toxaphene standard so that recognition by gas chromatography occurs primarily by retention time, and quantitation (detection to 1 ng) is generally accomplished by the method of alkaline dehydrochlorination, extraction and chromatography (Gomes, 1977). Metabolites are generally isolated by thin-layer chromatography or liquid chromatography.

III. TOXICOKINETICS

This chapter refers only to data relevant to warm-blooded vertebrates. Residue estimation in tissues after toxaphene exposure is generally based on the comparison of EC/GC traces of a test sample with the pattern of standards. Systematic errors in residue levels may exist because of the differential behavior of the >177 components of toxaphene relative to metabolism can render such comparisons inappropriate.

Absorption

Toxaphene is absorbed through the skin (especially if mixed with xylene), the lung, and the gut (IUPAC, 1979; IARC, 1979). The rates of absorption have not been documented. The available data are summarized in Table III-1.

Oral

One incident that illustrates toxaphene absorption involved toxaphene poisoning of the Tule Lake Basin of Northeastern California and Southern Oregon (Keith, 1965). Toxaphene, ~2.2 kg/ha, was used between 1956 and 1963 in areas on and off the Tule Lake and Lower Klamath Refuges, with heavy use occurring between 1957 and 1960 (>1000 ha treated/year) in Modoc County. Though many pesticides, including DDT, were also used in the area, the wildlife bird mortalities coincided with the significant use of toxaphene. Toxaphene was found in the carcasses and tissues of dead birds in 1960-1961 (0.3-15 mg/kg whole carcasses or organ composites) (see Chapter V for discussion on residue levels as linked to mortality). Keith (1965) also reported on the mortality of other birds (Robinson, 1959) in Nebraska and in Montana upland that were associated with toxaphene application.

TABLE III-1

Absorption Data for All Routes for Toxaphene

Absorption Route	Species	Exposure	Effect	Reference
Oral	birds	2.2 kg/ha	kills in N.E. California and southern Oregon; residues in carcasses	Keith, 1965
	birds	unknown	kills in Nebraska and Montana; residues in carcasses	Robinson, 1959
	bald eagle	unknown	death; residues in carcasses	Barbehenn and Reichel, 1981
	birds	unkown	kill; residues in carcasses	Pollock and Kilgore, 1978a
	range birds	unknown	kill; residues in carcasses	McEwen et al., 1972
	birds	unknown	kills in California, South Dakota, Texas and Arizona	U.S. EPA, 1977
	raccoon	unknown	kill; residues in carcass	U.S. EPA, 1977
	horse	unkown	poisoning, residues	Mount and Oehme, 1981
	pig	unknown	poisoning; residues	Buck et al., 1976 Mount et al., 1980 DiPietro and Haliburton, 1979
	cattle	various	poisoning; residues in tissues, milk	Claborn et al., 1963 Zweig et al., 1963

TABLE III-1 (cont.)

Absorption Route	Species	Exposure	Effect	Reference
Oral (cont.)	cattle	various	poisoning; residues in tissues, milk	Steele et al., 1978
				Steele and Mount, 1980
				Steele et al., 1980
Oral	humans	unknown	three fatal; two poisoning	McGee et al., 1952
				U.S. EPA, 1977
	humans	unknown	poisoning from contaminated fish; 8 fatalities; 44 poisonings	U.S. EPA, 1977,1980
	animals	various	LD_{50} values	see Table V-1
Inhalation	humans	unknown	bilateral hilar adenopathy with fine miliary lesions on lungs; acute pneumonia; blood eosinophilia; serum globulins increased	Warraki, 1963
	humans	various	no toxaphene residues (<0.1 mg/kg blood)	U.S. EPA, 1980
	animals	various	LD_{50}	Boots Hercules Agrochemicals, Inc., n.d.
Dermal	humans	unknown (lindane/ toxaphene)	illness	Pollock, 1958
	pig	various	nervous system disorder	DiPietro and Haliburton, 1979
	animals	various	LD_{50} values	see Table V-2

Toxaphene residues have been measured in the tissues of dead wild birds (U.S. EPA, 1979a). The carcass/brain residue ratio in bald eagles was 6.7, similar to that of cis-nonachlor, PCBs, oxychlordane and cis-chlordane in the same species (Barbehenn and Reichel, 1981). The ubiquity of the occurrence of toxaphene residues in birds implies that toxaphene absorption occurred by the oral and possibly percutaneous routes. In fact, Pollock and Kilgore (1978a) attributed two bird kills to eating of contaminated fish, and direct toxaphene exposure. McEwen et al. (1972) noted no change in bird numbers 1 week after spraying toxaphene on a range land, but during the 2nd week a significant decrease in bird numbers was observed and several bird carcasses contained 0.1-9.6 mg toxaphene/kg ww. Live birds in the area had carcass residues of around 0.4-1.0 mg/kg ww. Other cases of bird kills are summarized in Table III-1.

Poisoning incidents in domestic animals also demonstrate that absorption can occur by the oral route (see Table III-1). The U.S. EPA (1977) documented 38 specific cases of toxaphene exposure to wildlife and livestock between 1966 and 1975, including 10 for cattle, 3 for swine, 2 for horses and 1 for sheep. Animal oral LD_{50} values in toxicity experiments depend on the vehicle; i.e., readily absorbed oils like corn oil and peanut oil elicit low LD_{50} values, whereas carriers not readily absorbed like kerosene yield higher LD_{50} values (U.S. EPA, 1980) (see Chapter V).

Evidence for oral absorption in humans resulting in toxaphene poisoning is summarized in Table III-1 and treated fully in Chapter VI.

Inhalation

The relevant evidence for absorption by the inhalation route is summarized in Table III-1.

Dermal

Data in animal experiments (IUPAC, 1979) indicate that toxaphene can be absorbed through the skin. However, the toxicity is generally an order of magnitude less than acute oral LD_{50} values using the same carrier. The LD_{50} values are also very vehicle-dependent (see Chapter V). The values for the rabbit range from <250 mg/kg for toxaphene applied in solution to <1000-2000 mg/kg for toxaphene applied as a dust.

DiPietro and Haliburton (1979) reported an episode of toxaphene toxicosis in swine after topical application 36 hours earlier with ~10 times the prescribed toxaphene dose (300 mℓ of 61% toxaphene stock in 4 ℓ of H_2O) for sarcoptic mange.

Distribution

Since there are over 177 components of toxaphene that do not metabolize in the same manner, nor at the same rate; their identification can be difficult as a result of the change of the diagnostic EC/GC chromatogram from that of a reference chromatogram. This problem has been observed in milk analysis (Cairns at al., 1981) and analysis of bird organs (Haseltine et al., 1980). One solution is to dechlorinate the remaining toxaphene. This type of analysis has been performed by Chandurkar and Matsumura (1979b) for Toxicants B and C in rats and by Saleh and Casida (1978) for Toxicant B in rats. Another method is to study the metabolism of only the more toxic components of toxaphene. This has been performed by Chandurkar and Matsumura (1979b), and Saleh and Casida (1978). A third approach is to utilize radioisotopes. This has been done by Bush et al. (1978) using broiler chickens, by Chandurkar and Matsumura (1979a), Ohsawa et al. (1975), and Crowder and Dindal (1974) in rats. However, chromatographic confirmation is still necessary for the labeling experiments since the position of the label defines the significance of the results. Unfortunately, no single study has compared all methods simultaneously.

In general, toxaphene appears to be rapidly metabolized and its metabolites quickly excreted in most species, with fat as the preferred tissue of storage (IUPAC, 1979).

Distribution in Animal Tissues

Birds. A number of studies in avian species such as ring-necked pheasants (Genelly and Rudd, 1956a) and White Leghorn chickens (Bush et al., 1977, 1978), suggest that birds can metabolize toxaphene adequately, as long as the doses are not too high and they can tolerate steady ingestion of the chemical for a period of months without serious effects at the lower doses (Genelly and Rudd, 1956b; Page et al., 1978), with the exception of altered cartilaginous structures (Page et al., 1978). Young white pelicans and young water-fowl are more susceptible to toxaphene (Keith, 1966) (see Chapter V).

Concentrations of 0, 25, 100, 200 and 300 ppm toxaphene in the form of dietary mash were fed to adult ring-necked pheasants (10/sex) for up to 94 days (Genelly and Rudd, 1956a). The mean doses for 100 and 300 ppm levels were 4.74 and 9.12 mg toxaphene/bird/day, respectively. Organic chlorine residues were found in fat for all doses >4.74 mg/bird/day, and in liver at the 9.12 mg/bird/day dose. One bird at the 4.74 mg/bird/day dose showed high residues in the testes.

Toxaphene at 0, 0.5, 5, 50 or 100 ppm diet was fed to five groups of 90 1-day-old female White Leghorn chickens per group for up to 34 weeks (Bush et al., 1977). The pesticide, as Tox-S01-6, containing 59% active ingredient, was first dissolved into corn oil and then into a formulated diet. The level of toxaphene in excisable adipose tissue of 8-week-old birds increased with increasing dietary toxaphene as described by the following:

$$\text{mg toxaphene/kg body fat} = 3.4\ F + 33.5 \qquad r^2 = 0.725$$

where F is the level of toxaphene in the feed in ppm. This amount accounts for only 50% of the total toxaphene ingested. Accumulation in 32-week-old birds was less than in 8-week-old birds despite continued exposure. Bush et al. (1979) suggested that White Leghorns were able to metabolize or excrete toxaphene at a rate comparable with daily intake. Residues were found in the eggs of bird receiving dietary levels >5 ppm.

Toxaphene-^{36}Cl was utilized in a study by Bush et al. (1978) to assess the distribution of the labeled pesticide in broiler chickens. Concentrations of 0, 0.22, 0.40, 2.16 and 3.82 ppm toxaphene-^{36}Cl were administered to 1-day-old unsexed broiler chicks (three replicates of 30 birds each). Feed and water were supplied ad libitum under a regime of continuous incandescent light.

For toxaphene (T) in tissue (mg/kg ww) versus toxaphene in feed (ppm) after 8 weeks of continuous feeding (F), the following relationships were found:

Adipose: $T = 4.081\ F + 0.765$ $r^2 = 0.96$
Gizzard: $T = 0.1588\ F + 0.0140$ $r^2 = 0.7484$
Heart: $T = 0.1659\ F + 0.0565$ $r^2 = 0.9305$
Kidney: $T = 0.0942\ F + 0.0400$ $r^2 = 0.7512$
Liver: $T = 0.0355\ F + 0.0625$ $r^2 = 0.4528$
Breast Muscle: $T = 0.0472\ F + 0.0018$ $r^2 = 0.6269$
Leg Muscle: $T = 0.1307\ F + 0.0241$ $r^2 = 0.8669$

The intercepts in these equations are probably not different from zero.

Only in adipose tissue did appreciable amounts of toxaphene accumulate, but this still represented only 5% of the consumed toxaphene.

One-year-old black ducks of *Anas rubripes* were fed dietary levels of 0, 10 or 50 ppm toxaphene with the toxaphene dissolved in a volume of propylene glycol equivalent to 1% of the total diet (Haseltine et al., 1980). After hatching of eggs, the residues in ducklings fed on the same diets as their mothers were monitored at days 14 and 84 post-hatching. At day 14, the ducklings contained 6.3 and 20.8 mg toxaphene/kg bw for the 10 and 50 ppm dietary levels, respectively. At day 84, female ducks contained 7.7 and 28.0 mg/kg carcass. GC chromatograms of body residues were similar to the toxaphene standards.

Haseltine et al. (1980) also performed a one-generation study on the distribution of toxaphene in black ducks (15 pairs/group). Toxaphene was administered to newly hatched ducks (F_0 generation) continuously over 19 months in propylene glycol admixed so that the carrier constituted 1% of the duck mash feed. All ducklings obtained from the $F1_a$ and $F1_b$ breedings were fed the toxaphene containing diets until 12 weeks of age. The technical toxaphene concentrations in the diet were 0, 10 or 50 ppm. Toxaphene residues in liver, brain, blood and carcass were quantified by direct comparison of the total area under the GC curve with the total area under the standard toxaphene curve (Table III-2) or by comparing the area under two characteristic peaks with the area under the same peaks in the standard. The two methods did not agree, indicating that differential metabolism of the toxaphene components had occurred. Liver residues for the 50 ppm group at month 19 constituted only 12% of the cumulative dietary levels. Brain residues were higher in males (1-3 mg/kg ww) than in females (~0.5 mg/kg ww) exposed to 50 ppm. Blood levels of the 50 ppm group were not significantly higher than in the 10 ppm dosed birds. No brain residues were noted at the 10 ppm level. Carcass levels were higher for the 50 ppm treatment compared with 10 ppm.

Mammals. Lackey (1949a) administered doses of 4 g toxaphene in corn oil/day in a gelatin capsule to dogs and determined that brain tissues (and not fat) at day 106 after treatment showed increased organic chloride levels relative to unexposed animals. This was interpreted by the author to signify a storage of toxaphene or chlorinated derivatives in the brain but not in the fat. In sheep, steers and dairy cows, storage of toxaphene in fat did occur on repeated exposure (Diephuis and Dunn, 1949; Conley, 1952).

TABLE III-2

Distribution of Toxaphene Continuously Administered Orally to Black Ducks from Birth by Mixing Toxaphene in Propylene Glycol, which Constituted 1% of the Duck Mash*

Dose (ppm toxaphene in the diet)	Age	Gender	Generation	Mean Concentration (mg toxaphene/kg ww) in			
				Carcass	Liver	Brain	Blood
10	Week 2	M/F	$F1_a$	6.3±0.3	NM	NM	NM
			$F1_b$	3.1±1.3	NM	NM	NM
	Week 12	M/F	$F1_a$	7.7±0.9	NM	NM	NM
			$F1_b$	3.1±0.2	NM	NM	NM
	Month 19	M (6)	F0	3.1±0.6	0.40±0.08	<0.1	0.14±0.01
		F (6)	F0	3.0±0.04	0.40±0.07	<0.1	0.18±0.02
50	Week 2	M/F	$F1_a$	20.8±3.0	NM	NM	NM
			$F1_b$	7.0±1.4	NM	NM	NM
	Week 12	M/F	$F1_a$	28.0±3.5	NM	NM	NM
			$F1_b$	11.6±3.4	NM	NM	NM
	Month 19	M (6)	F0	28.0±5.7	6.1±1.3	1.4±0.6(5)	0.50±0.16
		F (6)	F0	11.1±1.4	2.2±0.4	0.68±0.45	0.29±0.03

*Source: Haseltine et al., 1980

NM = Not measured

Feeding toxaphene at 10 ppm in the diet of sheep and calves for 28 days revealed no detectable storage in the fat (Claborn, 1956). Feeding cattle 25 and 100 ppm in the diet for 16 weeks resulted in the appearance of 12 and 38 mg toxaphene/kg omental fat, respectively. Eight weeks after feeding stopped, storage in the 100 ppm diet group diminished to 0.5 mg/kg ww. Dermal spraying (0.5% solution) led to the appearance of toxaphene residues in cattle fat of 2.5 mg/kg ww after a single spray (Claborn, 1956). Analysis of fat 4 weeks after 1-2 sprayings of 0.5% solution at 2-week intervals showed toxaphene present in renal fat. Six weeks after the last spraying, no toxaphene could be detected in fat. Roberts and Radeleff (1960) suggested that meat from toxaphene-treated swine is safe for human consumption if the animals are slaughtered at least 6 weeks after being sprayed once or twice with a 0.05% solution of toxaphene.

Clapp et al. (1971) reported the results of a 12-week feeding study with 12 male and 12 female rats provided with feed containing 0, 2.33, 7, 21, 63 and 189 ppm toxaphene. TLC was used to assess levels in omental fat and liver. Levels in the liver were greatest at week 4, but fat levels increased continuously, although male rats tended to have maximum fat levels at week 8. Below the 21 ppm dose, residues in fat, liver and carcass were similar. Above this dose, fat residues were proportional to dose.

Crowder and Dindal (1974) studied the distribution of 20 mg technical ^{36}Cl-toxaphene/kg bw administered orally by stomach intubation in 0.5 mℓ of a peanut oil/gum acacia solution to 30-day-old Holtzman albino rats. The uptake of the label in various tissues from 3 hours to 20 days (Table III-3) showed that the highest concentrations in most tissues occurred in 12 hours.

TABLE III-3

Percent Uptake of Radioactivity in Various Rat Tissues and Organs Following a Single Dose of ^{36}Cl-Toxaphene (20 mg/kg) Administered in 0.5 mℓ Peanut Oil/Green Acacia*

Tissue	Days Post-Dosing												
	0.125	0.25	0.50	1	2	3	4	5	6	7	8	9	20
Blood Supernatant	0.64	1.2	2.4	1.3	0.60	0.36	-	0	-	0.18	-	0.09	0.06
Blood Cells	3.1	0	0	0.06	0.90	2.6	-	0	-	0	-	1.1	1.2
Stomach	3.7	19	77	2.0	0.63	0.61	-	0.39	-	0.16	-	0.12	0
Liver	0.33	1.1	2.3	0.50	0.31	0	-	0.01	-	0	-	0.48	0
Kidney	0.05	0.13	0.43	0.10	0.03	0.03	-	0.01	-	0	-	0.03	0
Fat	0.14	0.15	0.86	0.57	0.31	0.18	-	0.18	-	0.02	-	3.7	0.03
Testes	0.02	0.08	0.28	0.06	0.04	0.03	-	0.03	-	0.02	-	0.06	0
Brain	0.03	0.06	0.23	0.05	0.04	0	-	0	-	0	-	0.01	0
Muscle	0.93	1.6	5.3	1.3	0	0.65	-	2.4	-	0.40	-	0.14	0.81
Small Intestine													
First 2 cm	0.06	0.34	0.34	0.05	0.01	0	-	0	-	0	-	0.84	0.09
Last 2 cm	0.10	0.34	0.28	0.13	0.01	0.15	-	0	-	0	-	0	0
Large Intestine	0.19	0.60	1.2	0.19	0.08	0.02	-	0.03	-	0.04	-	0	0
Esophagus	0.04	0	0.04	0.03	0.01	0.01	-	0.02	-	0	-	0.03	0
Spleen	0.04	0.06	0.08	0.05	0.02	0	-	0.03	-	0.24	0	0.06	0
% Tissue Total	9.4	24.3	90.9	6.4	3.0	4.6	-	3.1	-	1.1	-	6.6	2.2
Feces				24	7.5	1.3	1.1	1.1	1.2	0.69	0.27	0.31	
Urine				1.5	3.2	2.9	2.4	1.8	1.2	1.2	0.54	0.72	
% Excretion Total				25	11	4.1	3.5	2.9	2.4	1.8	0.8	1.0	
Total % Recovered	9.4	24	91	31	14	8.7		6.0		2.9		7.6	

*Source: Crowder and Dindal, 1974.

All blood levels of labeled toxaphene peaked after 3 days. The data may indicate the distribution of toxaphene metabolites if metabolism is fast, rather than the distribution of toxaphene itself. In addition, if extensive dechlorination occurred the labeled Cl detected might represent inorganic Cl rather than organic Cl. The former appeared to be the case for the feces and urine. It appears that the ^{36}Cl is rapidly secreted with the stomach acids within 0.50 days after dosing, which implied that extensive metabolism had occurred.

Male Sprague-Dawley rats (250-290 g) were administered the ^{36}Cl-labeled compounds by stomach intubation at 0 and ~13 mg toxaphene/kg bw with 0.15 mℓ corn oil as the vehicle and with another 0.10 mℓ as rinse (Ohsawa et al., 1975). ^{14}C-toxaphene was similarly administered at 0, 8.5, 12.7 and 19.0 mg/kg. In the same study ^{14}C-labeled Toxicants A and B were administered to 200-225 g rats at 0 or 0.84 and 0 or 2.6 mg/kg bw, respectively, using 0.25 mℓ corn oil as the vehicle and also as rinse. The results given in Table III-4 show that toxaphene and Toxicants A and B were rapidly eliminated (high excretion percent) as metabolites, and that toxaphene did not remain as such in tissues to any great extent (^{14}C- and ^{36}Cl-toxaphene levels were not comparable). Most of the label resided in blood, fat, liver and kidneys. The ^{36}Cl-results are generally consistent with those of Crowder and Dindal (1974) although detectable levels in the blood and muscle were not observed. At day 14 between 2.2 and 6.6% of the ^{36}Cl-label should be found in the tissues (Crowder and Dindal, 1974) and >53% should have been excreted. Since 59% of the 19 mg ^{14}C-toxaphene/kg dose of toxaphene was excreted to day 14 in the Ohsawa et al. (1975) study, results of both studies are compatible. Toxicant A and B appeared to be

TABLE III-4

Distribution of Radioactivity in Various Rat Tissues and Organs at Day 14 Following a Single Dose of ^{36}Cl-Toxaphene, ^{14}C-Toxaphene, Toxicant A and B Administered in Corn Oil by Stomach Intubation[a]

Tissue	^{14}C-Toxaphene			^{36}Cl-Toxaphene	Levels (mg label/kg ww)	
	19 mg/kg	12.7 mg/kg	8.5 mg/kg	14.2 mg/kg	Toxicant A	Toxicant B[b]
Blood	NR	NR	0.14	NR	NR	0.07
Liver	0.30	NR	0.12	NR	NR	0.12
Kidney	NR	NR	0.17	NR	NR	0.09
Fat	0.78	NR	0.52	NR	NR	NR
Bone	NR	NR	0.02-0.09	NR	NR	<0.03
Brain	NR	NR	0.02-0.09	NR	NR	<0.03
Heart	NR	NR	0.02-0.09	NR	NR	<0.03
Lung	NR	NR	0.02-0.09	NR	NR	<0.03
Muscle	NR	NR	0.02-0.09	NR	NR	<0.03
Spleen	NR	NR	0.02-0.09	NR	NR	<0.03
Testes	NR	NR	0.02-0.09	NR	NR	<0.03
Urine[c]	31.8	25.4	21.3	49.1	28.3	26.7
Feces[c,d]	27.1	31.7	34.7	26.9	47.8	47.8
Breath $^{14}CO_2$[c]	NR	NR	1.2	NR	1.8	0.7
% Excreted[c]	58.9	57.1	57.2	76.0	68.5	75.2

[a] Source: Ohsawa et al., 1975

[b] At day 9

[c] Percent cumulative

[d] Methanol soluble fraction only

NR = Not reported

quickly dechlorinated. ^{14}C-Toxicant B was hardly detectable in most tissues at day 9 except for blood, liver and kidney (Ohsawa et al., 1975).

These results are somewhat different from those described in the earlier report of Clapp et al. (1971) in that no deposition in fat was observed, but the TLC technique utilized by the latter authors may not have been as specific as the techniques utilized by Crowder and Dindal (1974) and Ohsawa et al. (1975).

Male albino Sprague-Dawley rats were separately administered toxaphene (13 mg/kg bw); ^{14}C-toxaphene (1.5 mg/kg bw) and 3.1 mg/kg bw each of Toxicant B; 2-exo,5-endo,6-exo,8,9,10-hexachlorobornane (CAS RN 57981-29-0); and 2-endo,5-endo,6-exo,8,9,10-hexachlorobornane (CAS RN 65620-64-6); a mixture of the latter two compounds (0.52/0.95 mg/kg bw, respectively) using soybean oil (0.15 mℓ) as a vehicle for administration by stomach intubation; and 0.1 mℓ for the rinse (Saleh and Casida, 1978). GC analysis confirmed that all of these compounds underwent dechlorination, and that chromatograms derived from samples of fat did not resemble the toxaphene standard. This was not the case for the liver and feces. The amounts of Toxicant B and its metabolites in fat and liver at 7 and 72 hours after oral intubation are provided in Table III-5.

Peakall (1979) administered an acute single dose of 120 mg toxaphene/kg bw orally in a gelatin capsule to male rats. Four animals were sacrificed at 1, 5 and 15 days. The liver residues were 2.3, 4.2 and 5.7 mg/kg ww, respectively. The residues in brain were below detection limits, 2 and 2.7 mg/kg ww, respectively. When fed 2.4 mg toxaphene/kg bw/day, four animals

TABLE III-5

Distribution and Metabolism of Toxicant B (51775-36-1) at 7 and 72 Hours After Stomach Intubation of Sprague-Dawley Rats at 3.1 mg/kg bw*

Tissue	Time (hours) post dosing	Number of Rats	µg/kg ww of CAS RN			
			51775-36-1	65620-64-6	57981-29-0	64618-63-9
Fat	7	3	453±285	8±7	14±13	15±9
	72	5	335±44	13±5	34±8	14±2
Liver	7	3	8.6±4.1	5.9±1.9	10.2±2.3	3.4±0.9
	72	5	17.3±11.7	2.0±0.6	9.2±7.0	0.8±0.6

*Source: Saleh and Casida, 1978

were sacrificed at 1, 3 and 6 months. The liver residues were 28, 26 and 26 mg/kg ww, respectively. Brain residues were 9.3, 13 and 12 mg/kg ww, respectively. These results are suggestive that steady state plateau levels can be reached in liver and brain in chronic studies.

In another study, virgin female Sprague-Dawley rats (318±34 g) were used and pregnant animals were acutely administered 2.6 mg ^{14}C-toxaphene/kg bw in olive oil (0.1 mℓ) as a vehicle for oral gavage (Pollock and Hillstrand, 1982). Rat chow and water were available ad libitum. The amount of label in various organs, excreta, fetus and umbilical cord was measured. The results are provided in Table III-6. The urinary excretion of the label by pregnant animals up to day 5 compared well with that of nonpregnant females (Pollock and Hillstrand, 1982). The fecal excretion (28.3%) was slightly lower than that found in nonpregnant animals (35.7%). The levels in the fat for pregnant animals at day 5 (7.1-7.8 mg toxaphene/kg ww) also agreed well for levels in nonpregnant animals after 7 days (6.4 mg toxaphene/kg ww). All other tissues contained <10% of the level in fat. The carcass, cecum, small intestine and adrenal gland contained levels >0.5 mg toxaphene/kg ww at day 5. There were indications that metabolites resided in fat deposits and in the fetus, but these were not specifically identified. Transplacental transfer (<1% of the administered dose) also occurred, as did blood-brain barrier transfer. From Table III-6, the adrenal gland, carcass, cecum, abdominal fat, GI tract contents, kidney, large intestine, liver, ovaries and small intestines each contained over 1 mg toxaphene/kg ww at day 1. At day 3, only the adrenal gland, carcass, cecum, abdominal fat, GI tract contents and small intestines contained residues >0.5 mg toxaphene/kg ww.

TABLE III-6

Distribution of Radioactivity in Various Female Sprague-Dawley Rat Tissues and Organs Following a Single Dose of 2.6 mg ^{14}C-Toxaphene/kg bw Dose to Four Pregnant Rats Administered in Olive Oil (0.1 mℓ) by Oral Gavage[a]

Tissue	^{14}C-labeled Material (µg Toxaphene Equivalent/kg Wet Weight) at		
	Day 1	Day 3	Day 5
Adrenal gland	1092	577	625-731
Brain	185	63	57-62
Carcass[b]	1532	588	489-924
Cecum	6354	1225	1012-1136
Fat (abdominal)	7208	8474	7107-7845
Fetus	84	30	27-29
GI tract[c]	6534	1995	NM
Heart	349	90	71-92
Kidney	1195	317	303-347
Large intestine	2757	370	204-428
Liver	1210	397	363-384
Lungs	735	413	339-407
Ovaries	1054	175	469-484
Placenta	191	70	47-56
Small intestine	6890	629	486-1185
Spleen	171	49	50-73
Stomach	709	327	180-351
Umbilical	207	145	134-155
Uterus	154	99	56-216
Urine[d]	(8.9±2.0)	18.3	22.0
Feces[d,e]	(9.3±4.8)	23.8	28.3

[a] Source: Pollock and Hillstrand, 1982

[b] Contains blood and subcutaneous fat

[c] Contents of GI tract at termination

[d] Percent cumulative for four animals per day

[e] methanol soluble radioactivity

NM = Not measured

Distribution in Human Tissues

Apart from the instances noted in the absorption section, no residue data, or metabolites of toxaphene have been found in human tissue, even though 44 cases (including deaths) involving exposure of humans were documented between 1966 and 1975 in the United States (U.S. EPA, 1977). In an occupational study, exposure of 54 workers primarily by the inhalation or dermal routes did not generate detectable levels of toxaphene in the blood (>0.1 mg/kg blood) in spite of the fact that 49 of the personal air samples showed detectable amounts of toxaphene (U.S. EPA, 1980).

The above findings signify that toxaphene may either be quickly metabolized and transported in another form in humans, or that no absorption has occurred. In view of the many human and animal poisoning incidents involving toxaphene, it is more likely that toxaphene is metabolized quickly and then transported.

Metabolism

Toxaphene undergoes fast reductive dechlorination, dehydrochlorination and hydroxylation in mammals. Little is known about the mechanism of transport.

Toxaphene was thought to be slowly detoxified in such animals as sheep, steers and cows by excretion of ethereal sulfate and "glucuronate" (Conley, 1952). Crowder and Dindal (1974) in their distribution study of ^{36}Cl-toxaphene reported in the section on "Distribution in Animal Tissues" observed much redistribution of ^{36}Cl label with time. Most of the ^{36}Cl-labeled toxaphene was excreted (see Table III-3) within 6-7 days and little remained in the tissues.

Ohsawa et al. (1975) confirmed the observations of Crowder and Dindal (1974) with ^{36}Cl-toxaphene (see Table III-4). The only identified metabolite was chloride ion that appeared almost entirely in the urine and accounted for about half of ^{36}Cl-toxaphene eliminated. The dechlorination occurred mostly within two days. ^{14}C-toxaphene distribution as well as that of Toxicants A and B showed only a small amount unmetabolized in the feces (3% for ^{14}C-toxaphene) and that the metabolites probably included acidic materials partially or completely dechlorinated, and $^{14}CO_2$ (2% of ^{14}C-toxaphene). The ease of dechlorination appears to be shared by all components of toxaphene, even Toxicants A and B.

Khalifa et al. (1976) examined the reactions of toxaphene and Toxicants A and B with two iron (II) protoporphyrin systems, hematin reduced with sodium thiosulfate and microsomal cytochrome P-450 reduced with NADPH. They found that toxaphene reacts with reduced hematin in neutral aqueous medium to cleave about half of the C-Cl bonds, yielding derivatives with shorter retention times on gas chromatography and of reduced sensitivity for detection by electron capture. The system also converts Toxicants A and B to products formed by reductive dechlorination, dehydrochlorination and a combination of these reactions. Extensive metabolism of toxaphene and Toxicants A and B by rat liver microsomes required NADPH.

From these results it is clear that many toxaphene components, including Toxicants A and B, are converted by one or both of these iron (II) protoporphyrins to more polar derivatives (as evidenced by TLC). The studies with reduced hematin establish that toxaphene undergoes extensive dechlorination and that the products from Toxicants A and B determined by GC-CI-MS are formed by two or more of the following: reductive dechlorination, dehydrochlorination and vicinal chloride elimination.

Toxaphene has been shown to yield type I binding spectra with hepatic cytochrome P-450 of rats, mice, sheep and rabbits, which suggests that toxaphene may serve as a substrate for the hepatic microsomal MFO system (Kulkarni et al., 1975). Type II binding has not been observed.

In another rat metabolism study, Chandurkar and Matsumura (1979a) found that dechlorination and oxidative degradation by MFO involving cytochrome P-450 are active degradation mechanisms for toxaphene.

Toxaphene metabolism in chickens and 6 species of mammals (guinea pig, hamster, rabbit, mouse, rat, monkey) involves extensive dechlorination; and, in the case of rats, ~50% of the dose is excreted as chloride ion (Saleh and Casida, 1979; Saleh et al., 1977, 1979).

Saleh and Casida (1978) showed that the major products of Toxicant B produced by rat liver microsomes are CAS RN 57981-29-0 and 65620-64-6 in a 2:1. Table III-7 lists synonyms, chemical names and indexing terms for the major metabolites and degradation products of Toxicant B. These products were not detected under aerobic conditions or under aerobic or anaerobic conditions if NADPH was absent. These products were also found (see Table III-5) in fat, liver and feces in addition to CAS RN 64618-63-9 after oral

TABLE III-7

Identified Metabolites of Toxaphene Components

CAS RN MF	Chemical Abstracts Indexing Term (9CI) Synonym	Comments	Reference
57981-29-0 $C_{10}H_{12}Cl_6$	Bicyclo[2.2.1]heptane,2,3,6-trichloro-1,7,7,-tris(chloromethyl)-,(2-exo,3-endo,6-exo)-	Reductive dechlorination of toxaphene toxicant B in: reduced hematin system, bovine rumen fluid, rat liver microsomes, and rats *in vivo*	Saleh and Casida, 1978
	or		
	2-exo,5-endo,6-exo,8,9,10-hexachlorobornane	Fecal metabolite of toxaphene toxicant B in: chicken, mouse, rat, hamster, guinea pig, rabbit and monkey	Saleh et al., 1979
64618-63-9 $C_{10}H_{10}Cl_6$	Bicyclo[2.2.1]hept-2-ene,2,5,6-trichloro-1,7,7-tris(chloromethyl)-,5-(endo,6-exo)-	Reductive dehydrochlorination of toxaphene toxicant B in: reduced hematin system and rats *in vivo*	Saleh and Casida, 1978
	or		
	2,5-endo,6-exo,8,9,10-hexachloroborn-2,3-ene	Fecal metabolite of toxaphene toxicant B in: chicken, mouse, rat, hamster, guinea pig, rabbit and monkey	Saleh et al, 1979
65620-64-6 $C_{10}H_{12}Cl_6$	Bicyclo[2.2.1]heptane,2,3,6,-trichloro-1,7,7-tris(chloromethyl)-,(2-exo,3-endo,6-endo)-	Reductive dechlorination of toxaphene toxicant B in: reduced hematin system, bovine rumen fluid, rat liver microsomes, and rats *in vivo*	Saleh and Casida, 1978
	or		
	2-endo,5-endo,6-exo,8,9,10-hexachlorobornane	Fecal metabolite of toxaphene toxicant B in: chicken, mouse, rat, hamster, guinea pig, rabbit and monkey	Saleh et al., 1979
66157-70-8 $C_{10}H_{11}Cl_5$	Tricyclo[2.2.1.0^{2,6}]heptane,3,4-dichloro-1,7,7-tris(chloromethyl)-,	Reduced hematin conversion of toxaphene toxicant B	Saleh and Casida, 1978
	or		
	2,5-endo,8,9,10-pentachlorotricyclene		
70459-31-3 $C_{10}H_{10}Cl_8$	Bicyclo[2.2.1]heptane,2,3,5,5-tetrachloro-7,7-bis(chloromethyl)-1-(dichloromethyl)-(exo,exo)-	Toxaphene toxicant C metabolite in rat liver *in vitro*	Chandurkar and Matsumura, 1979b
	or		
	3,3,5,6-exo-8,9,10,10-octachlorobornane		

CAS RN = Chemical Abstracts Service Registry Number; MF - Molecular formula; 9CI - Ninth Collective Index, Chemical Abstracts administration by gavage of 3.1 mg Toxicant B/kg bw to male albino Sprague-Dawley strain rats. The ratio of CAS RN 57981-29-0 to 65620-64-6 was 1.8 in fat, and 1.7 in the liver 7 hours after administration. At 72 hours, the ratios were 2.6 and 4.6, respectively, and 2.5 in the feces. At least 7.4% of the administered dose was eliminated after 72 hours in rats by reductive dechlorination at the germinal dichloro group. The chromatograms of the residues in fat almost conformed to the toxaphene standard but chromatograms of liver and feces samples did not. The latter samples, however, did contain toxaphene components.

Chandurkar and Matsumura (1979a) reported that in the presence of NADPH, the liver microsomal fraction of rats metabolized ^{36}Cl-toxaphene and ^{14}C-toxaphene after 2 hours at 37°C to the extent of 22 and 9.5%, respectively. In the presence of glutathione (GSH), the extent of metabolized ^{36}Cl and ^{14}C toxaphene were 11 and 4.8%, respectively; and with cysteine as a cofactor, the respective values were 10 and 3.6%. Inhibitors of NADPH dependent mixed-function oxidases and of glutathione-S-transferases inhibited conversion of toxaphene to polar water-soluble metabolites. The results depended on which fraction was utilized from rat liver homogenates. For example, one NADPH dependent mixed-function oxidase inhibitor caused 100% inhibition of conversion by a 100,000xg supernatant fraction compared with 53% utilizing a 20,000xg supernatant fraction from the same homogenate. When Toxicants B (Cl$_8$) and C (Cl$_9$) were investigated in the 20,000xg supernatant under the same conditions as toxaphene, the extents of metabolism were ~73 and 56%, respectively in the presence of NADPH; the respective figures for glutathione were 31 and 47%. Whereas the extent of Toxicant C metabolism in the presence of NADPH was complete within 1 hour, the rate of Toxicant B conversion was constant with time to 3 hours. The control showed an ability to metabolize Toxicant B and C without added NADPH, Toxicant C being more prone in this regard. TLC then EC/GC revealed that different components in toxaphene were metabolized by the NADPH system as compared with the GSH system. Most of these metabolites were more polar than the parent compounds (TLC data), some being hydroxylic (esterification data), and some being still chlorinated (EC/GC data). Treatment of water soluble ^{14}C-toxaphene metabolites with β-glucuronidase in the presence of NADPH revealed an increase of 16.1% in ether extractable material originally present as glucuronides. Smaller increases of 7.8 and 8.6% were shown in the presence of β-sulfatase and dilute hydrochloric acid, respectively. Addition of UDP-glucuronic acid to a toxaphene/NADPH system increased the extent of metabolism 8-fold, but not in the additional presence of a NADPH dependent mixed-function oxidase inhibitor. These results show that oxidative metabolism is important for toxaphene. The water-soluble metabolites of GSH-fortified incubates are probably GSH-conjugates formed involving loss of a chlorine from the original toxaphene components.

In another report of Chandurkar and Matsumura (1979b), using the same 20,000xg supernatant system fortified with NADPH (see Chandurkar and Matsumura, 1979a), the major dechlorinated metabolite of Toxicant C *in vitro* was shown to have a molecular formula of $C_{10}H_{10}Cl_8$ (MS data). This compound has been designated as 3,3,5,6-exo-8,9,10,10-octachlorobornane (CAS RN 70459-31-3) (see Table III-7). Oxidation products of Toxicants B and C were not tertiary alcohols, but 4 products of Toxicant C were secondary alcohols, and one was a primary alcohol. No Toxicant B metabolites were primary alcohols. None of the secondary alcohols were diols. NADPH caused more Toxicant B metabolism than for Toxicant C, but no alcohol metabolites of Toxicant B could be found, thus being suggestive of the occurrence of reductive dechlorination rather than hydroxylative dechlorination. This may indicate different loci for metabolism of Toxicants B and C.

Pollock and Hillstrand (1982) reported that the metabolites measured in the fetus are apparently different from those measured in the fat of Sprague-Dawley dams based on TLC analysis. No metabolite identification was reported.

Elimination

In a study where White Leghorn chickens were fed 5, 50 or 100 ppm toxaphene in the diet for 34 weeks (Bush et al., 1977), the half-life of toxaphene in adipose tissue was 42, 25 and 20 days, respectively.

To determine the length of time necessary for depletion of ^{36}Cl-toxaphene residues from adipose tissue of broiler chickens, 15 birds from each of the replicates (45 birds/treatment group) were fed toxaphene-free feed from week 6 onwards (Bush et al., 1978). The half-life of toxaphene in adipose tissue was found to be 2.66, 2.76, 2.47 and 2.5 weeks for birds fed 0.22, 0.40, 2.16 and 3.82 ppm toxaphene in the diet, respectively. Data were obtained from three birds, each sacrificed at intervals of 2 weeks for 10 weeks.

Black ducklings were fed dietary levels of 0, 10 and 50 ppm technical grade toxaphene in propylene glycol (1% of a commercial duck feeder mash) until the egg laying season (1976) was over (Haseltine et al., 1980). A select number of eggs (F_0 generation) were allowed to hatch, and the F_1 generation ducklings were similarly exposed up to the next egg laying season (1977). In 1976, the six eggs from the F_0 generation ducks samples contained 4.4±0.4 and 23.5±1.9 mg/kg ww for the 10 and 50 ppm concentrations, using total area GC estimation. In 1977, the respective figures for the two levels were increased to 5.6±0.6 and 29.5±2.0 mg/kg ww. In 1977 the levels in both the eggs and adult carcases were similar except in females fed 50 ppm toxaphene. Carcass levels for females were 11.1±1.4 compared with egg levels of 29.5±2.0. The investigators suggest that residues are not concentrated in eggs, since eggs contained a similar toxaphene load as carcasses. Both adults and young birds can metabolize toxaphene and excrete enough that little accumulation takes place in the organs.

The half-life of ^{14}C- or ^{36}Cl-labeled toxaphene in rats after single oral doses appears to be less than a week, with most of the elimination occurring in the urine and feces (Crowder and Dindal, 1974; Ohsawa et al., 1975). Only a small portion of the urine and fecal metabolites are eliminated as glucuronide or sulfate conjugates (Chandurkar, 1977), in contrast to early findings by Conley (1952).

Ohsawa et al. (1975) found that 49% of ^{36}Cl-label was eliminated in the urine 14 days after administration of 14.2 mg ^{36}Cl-toxaphene/kg bw (see Table III-4); in the same time 25.4% of the label from a 12.7 mg ^{14}C-toxaphene/kg bw dose was eliminated in the urine. The bulk of the ^{36}Cl-label (90%) was chloride ion. After 14 days, 27% of ^{36}Cl-label was found in the feces. Thus, in 14 days, 76% of ^{36}Cl-label was found in the urine and feces. Subfractionated toxaphene gave essentially the same results. Unmetabolized toxaphene comprised 3.4% of the feces residues, and around 27% in urine and feces appear to be organics that are dechlorination products. Between 5 and 10% are completely dechlorinated.

The data for Toxicants A and B are compared with that for ^{14}C-toxaphene in Tables III-4 and III-8. In general, Toxicants A and B appear more readily eliminated but their dosages were lower than for toxaphene itself, and only one animal was tested. Unmetabolized compound constituted only 8.1 (Toxicant A) and 2.6% (Toxicant B) of fecal residues, respectively.

In a similar set of experiments (Saleh and Casida, 1978), a 1.5 mg ^{14}C-Toxicant B (CAS No. 51775-36-1)/kg bw dose was given to Sprague-Dawley rats by stomach tube with soybean oil as the vehicle. Only 0.2 ± 0.1% of unmetabolized Toxicant B was found in the feces at 0-72 hours, along with 2.1 ± 0.6, 5.3 ± 1.6 and 1.0 ± 0.3% of the administered label as CAS RN 65620-64-6, 57981-29-0 and 64618-63-9, respectively. The combination CAS RN 65620-64-6 and 57981-29-0 constituted 7.4% of the administered label, and constituted products of reductive dechlorination at the germinal dichloro group. When a mixture of CAS RN 65620-64-6 and 57981-29-0 (0.95/0.52 mg/kg) was administered to the same strain of rat, the feces contained 45-47% of the unmetabolized compounds collected up to 72 hours. The feces contained many peaks of shorter retention time than those in toxaphene standards.

Chandurkar and Matsumura (1979a) also utilized four male Sprague-Dawley albino rats to administer a dose of 15 mg ^{14}C-toxaphene/kg bw by a stomach tube (0.25 mℓ of corn oil). Fifty-seven percent of the label was excreted in the feces. Only 9% of the administered dose was found in the urine. On

TABLE III-8

Elimination of ^{14}C-Toxaphene, Toxicant A and Toxicant B Administered to Sprague-Dawley Rats by Stomach Intubation in Corn Oil Carrier[a]

Sample	Time After Treatment (days)	Label Eliminated (Percent of Administered Dose)			
		Toxaphene		Toxicant A	Toxicant B
		(8.5 mg/kg) 3 rats	(19 mg/kg) 2 rats	(0.84 mg/kg) 1 rat	(2.6 mg/kg) 1 rat
Urine	0-1	10.6	11.3	18.9	15.7
	1-2	6.3	11.8	6.0	6.2
	2-14	4.4	8.7	3.4	4.8
Feces	0-1	34.7[b]	27.1[b]	27.4	32.3
	1-2			6.0	9.7
	2-14			5.0	5.9
Breath $^{14}CO_2$	0-1	0.8	NM	0.8	0.5
	2-14	0.4	NM	1.0	0.2
Totals		57.2	58.9	68.5	75.2

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[a] Source: Ohsawa et al., 1975

[b] It is not clear if these figures are totals for days 0-14 or the same measurement for each time after treatment.

NM = Not measured

the average, 8.9% of the urine metabolites and 0.7% of the fecal metabolites were glucuronides; 9.5% of the urinary and 7.5% of the fecal metabolites were acid-hydrolyable products; only a small percentage (0.7%) of the total fecal metabolites are sulfate conjugates.

A single spray treatment of cows with 2 quarts of 0.5% toxaphene suspended in water did not produce detectable toxaphene in the milk (Leighton et al., 1952). Spraying cows twice at 3-week intervals with 2 quarts of 0.5% toxaphene emulsion or suspension resulted in peak levels of 0.51-0.82 mg/ℓ milk 1 or 2 days after the second spraying. Twenty-one days after spraying had ceased, the toxaphene concentrations returned to preexposure levels (Claborn, 1956). Spraying cows under exaggerated conditions (twice daily for 21 days with 57 mg of a 2% oil solution of toxaphene) resulted in peak residues of 0.11-0.50 mg/ℓ milk after ~3 days. Twenty-one days after spraying ceased, the concentration diminished to 0.06 mg/ℓ milk (Claborn, 1956).

Keating (1979) reported on toxaphene residues in cow milk from cows dipped once or twice weekly in Altik [0.25% w/w toxaphene plus 0.03% w/v Dioxathion (Delnav)]. Residues ranged from 27-45 mg/kg milk fat, with maximum residues found one day after dipping. When dipping was discontinued for a cow dipped in Altik once weekly, the levels decreased to 5 mg/kg milk fat after 19 days. Even at 32 days, toxaphene could still be detected (3.1 mg/kg).

Pollock and Hillstrand (1982) found that excretion in urine and feces for pregnant rats (Sprague-Dawley) is very similar to that in virgin female rats, although there was a large weight difference. The results for pregnant females are provided in Table III-6, which indicate a half-life of ~5 days.

Toxaphene was administered to cows continuously at increasing daily concentrations ranging from 1.7-12 ppm diet. Toxaphene levels were first detected in the milk of cows in the 4 ppm group after 3 weeks. Excretion equalled 7-8 mg toxaphene/ℓ milk in the 12 ppm group. When exposure was discontinued, the concentration in milk diminished promptly (Shaw, 1947). In cows fed contaminated hay for 16 weeks, the toxaphene levels in milk (50-410 mg toxaphene/ℓ) were proportional to the amount in the diet, with the average excretion ranging from 2.3-18.2 mg toxaphene/ℓ milk (Bateman et al., 1953). Claborn (1960) fed four groups of three cows each 20, 60, 100 and 140 ppm toxaphene in the diet, respectively. The excretion was proportional to the toxaphene concentration. Once exposure ceased, residues diminished to 13-17% within 1 week, followed by a slow excretion phase probably related to the mobilization of fat reserves.

Cows were fed 0-20 ppm toxaphene in the diet for 77 days; milk samples were analyzed twice weekly (Zweig et al., 1963). The results confirmed previous findings at higher feed levels that toxaphene residues declined rapidly after toxaphene exposure had terminated. Residues ranged from 0.043-0.179 mg toxaphene/ℓ milk, and were concentration-dependent. Following cessation of exposure, residues in milk decreased to undetectable levels after 2 weeks for levels lower than 10 ppm toxaphene. For the 20 ppm level, residues were detected 30 days after feeding contaminated diet was halted.

In another study involving 12 cows fed 5 ppm toxaphene in the diet (Cairns et al., 1981), toxaphene metabolites and components were found in milk fat with the EC/GC chromatograms being enriched in the components of shorter GC retention time. Six milk samples contained residues estimated by CI/SIM/GC/MS to range from between 7 and 280 mg/ℓ milk. An EC/GC study using toxaphene standards estimated the range to be 10-620 mg toxaphene/ℓ milk. The noncorrespondence of residues to intake may reflect the time of sampling since this was not specified. However, this does illustrate that the analytical methods for toxaphene may yield different answers when enrichment or depletion of selected compounds in toxaphene occurs.

A summary of elimination half-life data for various animal species is provided in Table III-9. Saleh and Casida (1979) reported that quick metabolism and elimination occurs in rats, mice, guinea pigs, hamsters, rabbits and chickens, with the monkey metabolizing toxaphene most extensively and rapidly, and chickens the least and slowest. The order of decreasing rate of metabolism is as follows: monkeys, rats, hamsters > mice, rabbits, guinea pigs > chickens for a dose of Toxicant B at 3 mg/kg bw. The data for toxaphene in Table III-9 are in general agreement with the elimination data for Toxicant B, with half-lives being <1 week for all mammals, including man.

Summary

Though no precise quantitative data are available, the symptoms of toxicosis as well as the presence of residues and metabolites after exposure in animals, birds and humans indicate that toxaphene can be absorbed through the skin (especially if mixed with xylene), the lung, and the gut.

TABLE III-9

Experimental or Calculated Half-Life of Toxaphene Elimination in Various Species

Animal	Dose[a] (carrier)	Compound	Route	Half-Life (days)	Reference
Broiler chicken (Hubbard-Hubbard)	0.22-3.82[b]	^{36}Cl-label	adipose	(18.2±1)	Bush et al., 1978
Cattle	100[b] for 16 weeks	Unlabeled	adipose	5-6(calc.)	Claborn, 1956
Cow	57 mg of 2% oil solution sprayed twice daily for 21 days	Unlabeled	milk	21	Claborn, 1956
Rat (Holtzman)	20 (peanut oil/ green acacia)	^{36}Cl-label	feces; urine	6-7	Crowder and Dindal, 1974
Male rat (Sprague-Dawley)	8.5-19 (corn oil)	^{14}Cl-label ^{36}Cl-label Toxicants A,B	feces; urine	<14	Ohsawa et al., 1975
Male rat (Sprague-Dawley)	0.52-0.95 (soybean oil)	^{14}Cl-label Toxicant B	feces	3	Saleh and Casida, 1978
Male rat (Sprague-Dawley)	15 mg (corn oil)	^{14}Cl-label	feces	4-5	Chandurkar and Matsumura, 1979a
Female rat (Sprague-Dawley)	2.6 (olive oil)	^{14}Cl-label	feces; urine	5	Pollock and Hillstrand, 1982
Humans	Catfish fillet 52 mg/kg fillet	Unlabeled	blood	6-7	U.S. EPA, 1978

[a] In mg toxaphene/kg body unless specified otherwise

[b] ppm diet

In birds (ring-necked pheasants, ducks and leghorn layers), toxaphene can be tolerated for months with the most sensitive effect being altered cartilaginous structures. Extensive dechlorination of toxaphene occurs with chlorinated organic residues occurring mostly in the fat. White Leghorns were able to metabolize or excrete toxaphene at a rate comparable with daily intake. In ducks, brain residues appeared at doses >10 ppm in the feed.

In mammals, storage of toxaphene in the fat has been reported in sheep, steers and dairy cows. Accumulation in cattle occurred at exposures to 25-100 ppm toxaphene in the feed for 16 weeks. In contrast, dogs appeared to store toxaphene derivatives preferentially in the brain. In male or female rats, fat levels of toxaphene were relative to concentrations >21 ppm in the diet. Studies with labeled toxaphene revealed quick dechlorination and subsequent elimination of toxaphene, Toxicant A and Toxicant B at doses ≤19 mg toxaphene/kg bw administered by gavage to male rats using corn oil as the carrier. Toxaphene derivatives resided in the blood, fat, liver and kidneys. When male rats were chronically dosed with 2.4 mg toxaphene/kg bw/day, plateau levels were found in the liver and brain after 1, 3 and 6 months. Transplacental transfer as well as blood-brain barrier transfer occurred in rats. The adrenal gland, carcass, cecum and abdominal fat of female rats contained toxaphene derivatives 3 days after acute dosing with 2.6 mg toxaphene/kg bw. Extensive dechlorination and rapid elimination of metabolites occurred also in guinea pigs, hamsters, rabbits, mice and monkeys. No residue data or metabolites of toxaphene in humans have been reported in the available literature.

In rats, successful detoxification appears to require NADPH and oxygen. Toxicants A and B underwent reductive dechlorination, dehydrochlorination, and vicinal chloride elimination. Involvement of the MFO system is suggested by the observation of Type I binding spectra with the hepatic cytochrome P-450 of rats, mice, sheep and rabbits, as well as the enhanced toxicity to mice of toxaphene in the presence of piperonyl butoxide. There also appeared to be a detoxification pathway dependent on GSH. Toxicant B resisted metabolism more than Toxicant C. Glucuronide (major) and sulfate (minor) conjugates have been detected as metabolites. Toxicant C also produced one primary and four secondary alcohols whereas reductive dechlorination predominated for Toxicant B. These data suggest that toxaphene components are not metabolized necessarily at the same loci in the microsomal system.

Elimination of products derived from toxaphene has been demonstrated to occur in the feces, urine and in expired air of rats. The fecal route appears to be the more predominant route than in urine. Milk and eggs also showed residues. The species order of decreasing elimination efficiency for Toxicant B is as follows: monkey, rat, hamster > mouse, rabbit, guinea pig > chicken.

IV. HUMAN EXPOSURE

This chapter will be submitted by the Science and Technology Branch, Criteria and Standards Division, Office of Drinking Water.

IV. SOURCES OF HUMAN EXPOSURE - TOXAPHENE

This chapter summarizes the available data on use, environmental fate, and occurrence to characterize the potential for exposure to toxaphene. A more extensive discussion of the information on toxaphene in the environment is presented in the draft document entitled "Occurrence of Pesticides in Drinking Water, Food and Air" (U.S.EPA, 1987).

Humans may be exposed to toxaphene from a variety of sources, including drinking water, food and ambient air. Individual exposure to toxaphene will vary widely based on factors such as where a person lives, works and travels, and what a person eats. Intake of toxaphene will be affected by age, weight and lifestyle.

The Exposure Estimates section of this chapter presents available information on the range of human exposure to and intake of toxaphene from drinking water, food and ambient air. It is not possible to provide an estimate of the number of individuals experiencing specific exposures from these sources. However, this section provides some insight into the sources' relative contributions.

A. Use/Environmental Fate

Toxaphene is a pesticide that was widely used during the 1960s and 1970s primarily on agricultural crops and livestock. In 1974, domestic production of the compound was estimated at 103 million pounds, with approximately 74 million pounds applied for agricultural uses (U.S. EPA, 1977). By 1982, domestic usage had declined to about 16 million pounds, with only 5.9 million pounds used on field crops (USDA, 1983). In November of the same year, EPA published in the Federal Register its intent to cancel or restrict registrations for products containing toxaphene. All major uses of toxaphene were cancelled (47 FR 53784)(USEPA 1982). In addition, all existing stocks of the compound could be used in certain specified situations until December 31, 1986. Formulations unsuitable for conversion to uses in any of the specified situations were discontinued after December 31, 1983. Toxaphene is no longer commercially available (Berg, 1986).

Once released into the environment, toxaphene is very persistent in both soil and surface waters. Toxaphene is relatively insoluble in water and binds readily with soil, consequently it is fairly resistant to leaching from the soil column. Biodegradation is not a factor in removing toxaphene from aerobic soils, with reported half-lives of up to 20 years. However, biodegradation in anaerobic soils can remove up to 50 percent of the compound in 6 weeks. Volatilization is the only important removal process for toxaphene in shallow soils. The persistence of low levels of toxaphene in surface waters over several years suggests that volatilization, biodegradation, hydrolysis, and adsorption to sediments are not rapid removal processes (Callahan et al., 1979). Toxaphene is a mixture of a number of chlorinated compounds; most of these compounds are expected to bioaccumulate.

B. Occurrence

Drinking Water

Under the interim drinking water regulations, all community drinking water suppliers are required to monitor for toxaphene. Since the regulations took effect, no system has reported a violation of the 5 ug/L interim standard (FRDS, 1984).

The 1978 National Rural Water Survey, as well as several regional surveys, provide information on the occurrence of toxaphene in public drinking water supplies. According to available information, toxaphene concentrations did not exceed the minimum quantification limit of 0.17 ug/L in the 71 groundwater systems tested (U.S. EPA, 1984). Regional studies of toxaphene in the 1970's have generally reported that toxaphene, when it was detected, did not occur at levels greater than 0.1 ug/L (U.S. EPA, 1987). Current toxaphene concentrations in drinking water supplies are believed to be even lower than in the 1970's, due to the restrictions placed on the use of the compound in 1982 and its later removal from production.

Diet

Although toxaphene is no longer used, the pesticide is still expected to occur at low levels in many foods because of its past widespread use and persistence in the environment. Toxaphene has been detected in the FDA market basket surveys (FDA, 1986). Surveys conducted between 1982-1985 reported finding daily intakes of toxaphene for 25-30 year old males and females of 0.576 and 0.369 ug/day, respectively. Dietary levels are expected to decrease in the future because of the discontinuation of the use of toxaphene.

Air

According to U.S. EPA (1980) and Arthur et al. (1976), particularly high toxaphene concentrations were reported, in several agricultural regions of the southern United States in the early and mid 1970's, with levels ranging up to 1.747 ug/m^3. The highest reported toxaphene level was 8.7 ug/m^3 in Arkansas in 1970 (Kutz et al., 1976). In the 1970's, toxaphene was widely used as an agricultural insecticide, and the data from the 1970's reflect this use. Due to the discontinuation of toxaphene in the last 5 years, ambient air levels today are likely to be very low.

C. Exposure Estimates

The following table summarizes the current exposure levels of toxaphene in drinking water, food and air. The drinking water levels are based on the range of the levels found in the regional studies in the 1970's. The estimated daily intake levels were made based on the assumption of an intake of 2 liters per day of drinking water. Current levels are expected to be lower. The dietary exposures are taken from an average of several total diet studies taken from different regions of the United States from 1982 to 1985.

This number is a reasonable estimate of the average dietary intake of adult males between the ages of 25-30 years old. Some individuals would be expected to have a higher intake than the average.

IV-5

Exposure Estimates for Toxaphene

	Reported Exposure Levels (low-high)	Estimated Adult Intake
Drinking Water	0-<0.1 ug/L	0-<0.2 ug/day
Diet	--	0.6 ug/day
Air	Negligible	Negligible

The current available information on occurrence of toxaphene is insufficient to determine the national distribution of intake by any of the three routes. However, EPA believes that intakes from diet will generally be less than 0.6 ug/day and that air exposure to toxaphene is expected to be negligible. If toxaphene does occur in drinking water at levels of more than a few tenths of a ug/L, it is likely to be the major source of intake. However, for the majority of individuals in the United States diet is the major source.

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V. HEALTH EFFECTS IN ANIMALS

Acute and Subchronic Toxicity

Current studies of acute toxicity resulting from exposure of experimental animals to toxaphene can be divided into two main groups: exposure to unfractionated (technical grade) toxaphene and exposure to specific toxaphene components or subfractions, e.g., Toxicants A and B. Tables V-1, V-2 and V-3 summarize the LD_{50} values observed in each of these experimental situations. Greater than 10-fold differences in toxicity have been documented for various toxaphene fractions or components that differed from each other in chemical composition, polarity and solubility (Pollock and Kilgore, 1978a,b).

Information on the acute oral toxicity of unfractionated toxaphene to laboratory animals is summarized in Table V-1. In cases of acute intoxication, toxaphene like most chlorinated hydrocarbon insecticides, appears to act as a CNS stimulant. However, unlike DDT, toxaphene does not significantly affect conduction in the rat superior cervical ganglion (Whitcomb and Santolucito, 1976). Effects of toxic exposures in humans (hypersensitivity, tremors and convulsions) are similar to those observed in both rats and dogs (Lehman, 1951). Along with convulsions, hyperreflexia has also been noted in dogs (Lackey, 1949a), and rats (Boyd and Taylor, 1971).

It is important to note that humans who ingest a protein-deficient diet may represent a susceptible population. Rats fed a protein-deficient diet were more susceptible to toxaphene poisoning than rats fed laboratory chow, LD_{50}, 80 ± 19 and 220 ± 33 mg/kg bw, respectively (Boyd and Taylor, 1971).

TABLE V-1

Acute Oral Toxicity of Technical Toxaphene (CAS RN 8001-35-2) to Laboratory Mammals

Species	Vehicle	LD_{50} (mg/kg bw)	Reference
Rats:			
Unspecified strain	corn oil	60	Lehman, 1948; Boots Hercules Agrochemicals, Inc., n.d.
Wistar, male, (3-4 weeks, 50-60 g) low protein diet optimal diet	cottonseed oil	 220 ± 33^a 80 ± 19 293 ± 31^a	Boyd and Taylor, 1971
Sherman, male, (>90 days, >175 g)	peanut oil	$90(67-122)^b$	Gaines, 1960
Sherman, female, (>90 days, >200 g)	peanut oil	$80(70-91)^b$	Gaines, 1960
	peanut oil	40	Shelanski and Gellhorn, n.d.
	corn oil	120-125	Shelanski and Gellhorn, n.d.
Sprague-Dawley, male (24 days, 70 g)	olive oil (i.p.)	228	Pollock et al., 1983
Unspecified strain, male	unspecified	270	Kuz'minskaya et al., 1980
Mice	corn oil	112	Boots Hercules Agrochemicals, Inc., n.d.
	unspecified oil	80	Rico, 1961

TABLE V-1 (cont.)

Species	Vehicle	LD_{50} (mg/kg bw)	Reference
Mice			
Swiss-Webster, male	corn oil	112	Fattah and Crowder, 1980
Cats	peanut oil	25-40^c	Treon et al., 1950
	unspecified oil	100	Rico, 1961
Dogs	corn oil	20-25	Lackey, 1949a
	kerosene	>400	Lackey, 1949a
	peanut oil	>30^c	Treon et al., 1950
	deodorized kerosene	>450^c	Treon et al., 1950
Rabbits	peanut oil	75-100^c	Treon et al., 1950
	deodorized kerosene	250-500^c	Treon et al., 1950
Guinea pigs	corn oil	270	Boots Hercules Agrochemicals, Inc., n.d.
	kerosene	365	Boots Hercules Agrochemicals, Inc., n.d.
	unspecified oil	80	Rico, 1961
Hamsters:			
Syrian Golden, male and female, 6 weeks	unspecified	200	Cabral et al., 1979
Calves:			
Mixed-breed, 136-232 kg	60% solution, unspecified, (Cooper-Tox Emulsifiable Concentrate) diluted with 2 ℓ water	62	Steele et al., 1980

TABLE V-1 (cont.)

Species	Vehicle	LD_{50} (mg/kg bw)	Reference
Cattle	grain	144	Boots Hercules Agrochemicals, Inc., n.d.
Sheep	xylene	200	Boots Hercules Agrochemicals, Inc., n.d.
Goat	xylene	200	Boots Hercules Agrochemicals, Inc., n.d.
Horse	unspecified	80[c]	Rico, 1961
Monkey: female	peanut oil saline (i.v.)	7.5-10	Treon et al., 1950
Pheasant: Unspecified strain	unspecified	40	Metcalf, 1981
Mallard: *Anas platyrhynchos*	unspecified	71	Hudson et al., 1972
Hen: Unspecified strain	unspecified	100	Rico, 1961

[a] Standard error of the mean

[b] 95% confidence interval

[c] Minimum lethal dose

TABLE V-2

Acute Dermal LD_{50} Values for Toxaphene in Laboratory Mammals

Species	Vehicle	LD_{50} (mg/kg bw)	Reference
Rats			
Sherman, male (>90 days, >175 g) unfasted	xylene	1075 (717-1613)*	Gaines, 1960, 1969
Sherman, female, (>90 days, >175 g)	xylene	780 (600-1014)*	Gaines, 1960, 1969
Rats	xylene	930	Boots Hercules Agrochemicals, Inc., n.d.
Rats, male	unspecified	940	Kuz'minskaya et al., 1980
Rabbits	dust	~4000	Boots Hercules Agrochemicals, Inc., n.d.
Rabbits	peanut oil	~250	Boots Hercules Agrochemicals, Inc., n.d.

*95% confidence interval

TABLE V-3

Acute Toxicity of Technical Toxaphene Components Administered Intraperitoneally to Mice

Toxaphene Component (CAS RN)	Species/Strain	Vehicle	LD_{50} (mg/kg bw)	Reference
2,2,5-*endo*-6-*exo*-8,9,10-heptachlorobornane (51775-36-1)	mice/Albino male	unspecified	75	Saleh et al., 1977
2,2,5-*endo*-6-*exo*-8,9,10-heptachlorobornane (51775-36-1)	mice/unspecified	unspecified	6.6	Khalifa et al., 1974
2,2,5-*endo*-6-*exo*-8,9,9,10-octachlorobornane (58002-19-0)	mice/unspecified	unspecified	3.1	Khalifa et al., 1974
Technical toxaphene	mice/Swiss-Webster, male	dimethyl sulfoxide	33(27-41)*	Pollock and Kilgore, 1980
	rats/Sprague-Dawley, male	olive oil	228	Pollock et al., 1983

*95% confidence limits

Clinical signs of depression and stimulation of the CNS were the same in both groups; however, signs appeared earlier and at lower concentrations in protein-deficient rats. Pathological effects included cloudy swelling and congestion of the kidneys, fatty degeneration and necrosis of the liver, and decreased spermatogenesis. Mehendale (1978) reported that toxaphene (100 ppm in the diet for 8 days) inhibited hepatobiliary function in rats.

The acute dermal toxicity of toxaphene is summarized in Table V-2. Toxaphene appears to be somewhat less toxic when administered dermally although toxicity appears to be carrier-dependent. In rats the ratio of dermal to oral LD_{50}s is 12 (1075/90) for males and 9.8 (780/80) for females (Gaines, 1960, 1969). Dermal LD_{50}s for rats range from 780-1075 mg/kg bw (Gaines, 1960, 1969; Boots Hercules Agrochemicals, Inc., n.d.).

A 40% technical toxaphene dust (3-4 g/m³) killed around half of an exposed group of rats after 1 hour (Boots Hercules Agrochemicals, Inc., n.d.). Assuming that a 200 g rat inhales 200 mℓ of air per minute and absorbs 100% of the inhaled dose, a 3 g/m³ concentration for 60 minutes would equal 72 mg/kg bw.

Repeated (14 applications) administration of a 20% solution of toxaphene in kerosene to the eyes of rabbits (0.01 mℓ) and guinea pigs (0.05 mℓ) caused mild irritation to the eyelids with loss of hair of the eyelids. There was no injury to the eye and condition on the lids cleared completely in 10 days (Boots Hercules Agrochemicals, Inc., n.d.).

Hyde et al. (1978) compared the acute electrophysiological effects of representative organochlorine and organophosphate insecticides on laboratory rats. Concurrent neurological, respiratory and cardiac involvement were primary concerns. Each of 14 male Sprague-Dawley rats (240-280 g) was equipped with dural electrodes to record spontaneous cortical electroencephalograms. The animals were given a minimum of 7 days to recover from electrode implantation and were randomly assigned to 1 of 7 treatment groups, 2 rats/group. Treatments consisted of separate lethal i.p. injections of each of three organochlorine pesticides, including 70 mg toxaphene/kg bw and three organophosphates emulsified in a Tween-80 isotonic saline solution. Controls received 0.5 mℓ of the vehicle only. Total percentage changes in cardiac and respiratory rates during exposure to toxaphene (expressed as percentage change from pretreatment to late stage of toxicity) were ECG rates, +17.5 and respiration, +56.5. The distortion of spontaneous cortical potentials recorded during toxaphene exposure revealed a cerebral sensitivity.

Toxaphene is used extensively for external parasite control in domestic animals. The maximal nontoxic concentration for topical application in adult swine is a 0.5% solution. DiPietro and Haliburton (1979) reported signs of a CNS disorder in about 40 of a herd of around 150 feeder pigs. Thirty-six hours earlier the affected animals had been treated for sarcoptic mange with a toxaphene mixture applied at ~10 times the manufacturer's labeled dosage (300 mℓ of 61% toxaphene stock in 4 ℓ H_2O). One day after the treated pigs were sprayed with warm water, severely affected pigs had improved markedly. By day 5 the animals appeared normal.

Deaths and abnormal behavior in a herd of 50 crossbred beef cows in good body condition were attributed to an ingestion of toxaphene-contaminated feed (Braun et al., 1980). Levels of 11-35 mg toxaphene/kg hay were detected in four samples from pastures where the animals grazed.

With the objective of determining tissue levels of toxaphene following exposure to a single oral dose of toxaphene, Steele et al. (1980) exposed heifer calves ranging in weight from 136-232 kg to the following doses in mg/kg bw (number of animals exposed to each dose in parentheses): 50 (6), 100 (7), 150 (6). Toxaphene originally available as a 61% solution was diluted with ~2 ℓ of water. Surviving animals were sacrificed after 7 days. In the low dosage group, there were 2/6 deaths after 4 days; in the 100 mg/kg exposed group there were 6/7 deaths within 4.5-42 hours, and in the high dose group, there were 5/6 deaths within 21 hours to 5 days. Clinical signs of toxicity included apprehension and hyperexcitability, followed by anterior, then posterior, muscle fasiculations. Terminal stages were characterized by generalized muscle twitching and clonic-tonic convulsions. When toxaphene tissue levels were determined, it was observed that while kidney and brain toxaphene levels were not diagnostically significant, liver residues of toxaphene >4 mg/kg ww were associated with lethality for the 7-day observation period (Steele et al., 1980). By contrast, brain levels of toxaphene may be indicative of acute toxicity; as a result of inadvertent dermal exposure in swine, ≥4 mg/kg ww in the brain was found to constitute a lethal level, and 2 mg/kg was associated with clinical signs of toxicity (DiPietro and Haliburton, 1979). A summary is provided in Table V-4. Acute intoxication of horses has also been reported following ingestion of alfalfa contaminated with DDT and toxaphene (level not reported) (Nazario and Capellaro, 1980).

TABLE V-4

Residue Concentrations in Dead Birds and Animals

Animal	Organ	Residue Range[a] (mg/kg ww)	Reference
Female bat, *Tadarida brasiliensis Mexicana*) (1/1)[b]	carcass	2	Geluso et al., 1981
Rabbit (2/31)[b]	carcass	1.2-12.4	Causey et al., 1972
Deer (3/22)[b]	carcass	1.7-8.7	Causey et al., 1972
Heifer calves (6/7)[b]	liver	4 mg/kg was critical level for acute exposure	Steele et al., 1980
Swine	brain	4 mg/kg was critical level for acute exposure	DiPietro and Haliburton, 1979
Bald eagle (8/49)[b]	brain	0.13-1.2 (1975)	Kaiser et al., 1980
(20/49)[b]	carcass	0.06-2.5 (1975)	
(7/50)[b]	brain	0.05-0.31 (1976)	
(11/50)[b]	carcass	0.05-0.55 (1976)	
(13/69)[b]	brain	0.06-2.7 (1977)	
(22/69)[b]	carcass	0.06-0.88 (1977)	
Great blue heron (9/35)	brain	0.17-0.82 (1972-1978)	Ohlendorf et al., 1981
(6/36)[b]	carcass	0.11-0.50 (1972-1978)	
Cattle egret (3/3)[b]	brain	0.28-0.36 (1978)	
(2/3)[b]	carcass	0.11-0.16 (1978)	
Great egret (1/1)[b]	brain	0.54 (1978)	
(1/1)[b]	carcass	0.58 (1978)	

[a] Range in positives

[b] Positives in total birds and animals examined

The acute toxicity of toxaphene components in mice is listed in Table V-3. In male Sprague-Dawley 24-day-old rats, the less polar toxaphene fraction, R_f 0.35-0.85, and a more polar fraction, R_f 0-0.57, is included in this table and exhibited LD_{50} values of 217 and 266 mg/kg bw, respectively, compared with toxaphene, R_f 0-0.85, LD_{50} 228 mg/kg. The route of administration was i.p., and the solvent used in the toxicity determination was olive oil (Pollock et al., 1983).

In a comparative study certain polychlorobornanes that are known to be components of toxaphene have been examined for structure-toxicity relationships (Saleh et al., 1977; Saleh and Casida, 1979); using 2,2,5-endo, 6-exo, 8,9,10-heptachlorobornane as a standard with a relative toxicity of 100 (LD_{50} i.p. 75 mg/kg bw in male albino mice). An enhanced acute toxicity was associated with the introduction of a single chloro group in the 8- or 9-position, and to a lesser extent in the 5-exo-position. Pretreatment of mice with piperonyl butoxide (which modifies cytochrome P-450-mediated oxidative or reductive detoxification mechanisms) was associated with lower acute LD_{50} values (2- to 8-fold) for the heptachlorobornane compound and a mixture of its 8- or 9-monochloro derivatives. Acute toxicity was reduced relative to the standard when monochloro groups were introduced in either the 3-exo or 10-positions, but the toxicity of the 10-chloro derivative was also enhanced 2- to 8-fold by pretreatment of mice with piperonyl butoxide. Dehydrochlorination of the hexachlorobornenes with apparent removal of the 6-chloro group yielded a hexachlorobornane with only slightly increased acute toxicity relative to the parent compound.

LD_{50} values for 20-29 g male Swiss-Webster mice were compared for toxaphene and for three toxaphene fractions dissolved in dimethyl sulfoxide, and administered i.p. Only one fraction, with an LD_{50} of 20 mg/kg bw (19-21, 95% confidence intervals), was more toxic than toxaphene (33 mg/kg) itself (Pollock and Kilgore, 1980).

Table V-5 summarizes the effects of acute and subchronic oral administration of toxaphene to laboratory mammals. Except for convulsions observed in dogs given 10 mg/kg bw/day (Lackey, 1949a), few of the exposures detailed in Table V-5 resulted in clinical signs of toxaphene poisoning.

Lackey (1949a) exposed dogs of an unreported breed and sex both acutely and subchronically to toxaphene. Dosing was performed by stomach tube with the toxaphene dissolved in corn oil or kerosene for acute experiments and corn oil administered as a single dose daily in a gelatin capsule for longer term exposures. None of the three animals receiving a single dose of 5 mg/kg bw in corn oil developed convulsions as compared with 4/5 treated with 10 mg/kg. At doses $\geq$15 mg/kg the convulsions were accompanied by increasing mortality. Of those treated with toxaphene dissolved in kerosene, the minimum dose at which animals had convulsions was 75 mg/kg; none were recorded for two dogs dosed with 25 mg/kg or one dog receiving 50 mg/kg. These effects may actually indicate a possible vehicle effect.

Daily treatment with 5 mg/kg toxaphene resulted in the onset of convulsions after a few days. For subchronic treatment the dosage was therefore decreased to 4 mg/kg bw. Two dogs were treated daily at this level for 44 days and two for 106 days. One dog treated for 106 days and both dogs

TABLE V-5

Acute and Subchronic Oral Toxicity of Toxaphene

Species	Number of Animals	Vehicle	Duration	Dose (mg/kg/day or ppm in the diet)	Response	Reference
Mice, albino and wild strains	5-18	diet	Several weeks or months	50 mg/kg/day (250-480)*	Changes in blood chemistry and urine protein	Bäumler, 1975
Albino rats	12M, 12F at each level	diet	4, 8 and 12 weeks	2.33 7.0 21.0 63.0 189.0	No effect on weight, liver cell histology	Clapp et al., 1971
Rats	12M, 12F	diet	12 weeks	189*	No apparent adverse effects	Clapp et al., 1971
Rats	unknown	NS	7 months	1.2-4.8 mg/kg/day	Temporary change in blood chemistry	Grebenyuk, 1970
Rats, Sherman, male and female, 100 g	6M, 6F at each level	diet	2-9 months	50 and 200*	Questionable liver pathology	Ortega et al., 1957
Rats	4	diet	8 days	100*	Decreased bile production	Mehendale, 1978
Rats, Osborne-Mendel	5M, 5F at each level	acetone	6 weeks	160* 320* 640* 1280* 2560*	No deaths and mean weight gains unaffected (1280). 2 deaths (both F) and mean weight gains of survivors not adversely affected (2560)	NCI, 1979
Rats, Osborne-Mendel	5M, 5F at each level	acetone	6 weeks	1280* 2560* 5120*	Two deaths (1M, 1F) at 2560	NCI, 1979

TABLE V-5 (cont.)

Species	Number of Animals	Vehicle	Duration	Dose (mg/kg/day or ppm in the diet)	Response	Reference
Mice, B6C3F1	5M, 5F at each level	acetone	6 weeks	40* 80* 160* 320* 640* 1280*	2 deaths (1 M, 1 F) and mean weight gains unaffected (320). 6 deaths (4 M, 2 F) (640)	NCI, 1979
Dogs	3	corn oil	1 exposure	5 mg/kg/day	No convulsions	Lackey, 1949a
	5	corn oil	1 exposure	10 mg/kg/day	Convulsions in 4/5 animals	Lackey, 1949a
	2	corn oil	44 days	4 mg/kg/day	Questionable liver pathology: renal tubular degeneration; sporadic convulsions	Lackey, 1949a
	2	corn oil	106 days	4 mg/kg/day	Questionable liver pathology: renal tubular degeneration; sporadic convulsions	Lackey, 1949a

*ppm toxaphene in the diet

NS - not specified; M - male; F - female treated for 44 days lost weight during the course of exposure. Convulsions were reported to occur only occasionally. There were no changes in complete blood counts throughout the experiment and no gross pathological changes were found. Upon histopathology the liver showed generalized hydropic degenerative changes, which appeared to be reversible, and degenerative changes in the parenchyma.

In both the Lackey (1949a) study (using dogs) and the Ortega et al. (1957) study (using rats) changes in liver histology were noted in the animals dosed with toxaphene. Morphologically, these changes appeared as vacuoles of plasma with occasional red blood cells appearing within hepatic cells. This condition, referred to as hydropic accumulation, is distinct from fatty degeneration. In neither rats nor dogs was hydropic accumulation associated with the destruction of hepatic cells. Ortega et al. (1957) also noted occasional masses of red blood cells invading the cytoplasm of liver cells in areas of centrolobular-cell hypertrophy and margination was observed in 50% of the rats fed a diet containing 200 ppm toxaphene from 2-9 months. Similarly, 27% of rats fed 50 ppm showed this same effect. No control rats were examined however.

In addition to liver damage, Lackey (1949a) documented widespread degeneration of the tubular epithelium of the kidney, occasionally accompanied by inflammation of the pelvis of the kidney for dogs fed 4 mg/kg bw/day for 44 and 106 days. Similar pathological changes were seen in dogs and rabbits who survived prolonged dermal exposures [200-600 mg/kg bw for up to 32 days (dogs); 100-1000 mg/kg bw for up to 30 days (rabbits)] to toxaphene (Lackey, 1949b). For dogs, the lowest lethal dose was 600 mg toxaphene/kg bw; for rabbits, the lowest lethal dose was 600 mg/kg bw when dissolved in mineral oil or dimethyl phthalate, with the highest nonlethal dose being 500 mg/kg bw when applied as a dust for 5-14 applications (Lackey, 1949b). Ortega et al. (1957), however, did not note any pathological changes attributable to toxaphene in the kidneys of rats fed a 50 or 200 mg toxaphene/kg diet for up to 9 months.

Allen et al. (1983) studied the effects of ingested toxaphene on the immune response of female Swiss-Webster mice. A total of 130 8-week-old mice were given feed containing 10, 100 or 200 ppm toxaphene for 8 weeks (acetone solvent). Humoral antibody production, IgG antibody formation, was suppressed in mice receiving the 100 and 200 ppm doses. Cell-mediated immune responses were not affected by toxaphene treatment.

Clapp et al. (1971) fed technical grade toxaphene to groups of 12 male and 12 female albino rats (144 total) in their diets for 4, 8 and 12 weeks at levels of 0, 2.33, 7, 21, 63 and 189 ppm. Four animals from each group were sacrificed at each time interval. Two male rats died during the test period but their deaths did not appear to be caused by toxaphene levels in the diets. Toxaphene had no measurable adverse effects on physical appearance, gross pathology, weight gain or liver cell histology of any of the experimental animals.

Cattle and sheep have been fed toxaphene at concentrations as high as 320 ppm in hay for 134-151 days. Central nervous stimulation with muscle tremors occurred in steers at the highest dose, but not in sheep receiving a similar dose. There appeared to be no abnormal histopathology or blood chemistry (Boots Hercules Agrochemicals, Inc., n.d.).

Guinea pigs suffered 73% mortality when solutions of 20% toxaphene in mineral oil, equivalent to a dose of 332 mg toxaphene/kg bw/day, was administered for 14 days (Boots Hercules Agrochemicals, Inc., n.d.).

Feeding studies were conducted to estimate the maximum tolerated doses of toxaphene in Osborne-Mendel rats and B6C3F1 mice for a National Cancer Institute study of carcinogenicity (NCI, 1979). Toxaphene was dissolved in acetone and added to the feed. Corn oil was also added as a dust suppressant in an amount equal to 2% of the final weight of feed. Treatment groups, consisting of 5 male and 5 female animals in each group, were given food with or without toxaphene for 6 weeks, and they were observed for another 2 weeks.

For rats, toxaphene was added to the feed in 2-fold increasing concentrations, ranging from 160-2560 ppm feed. A second study was performed on male and female rats at toxaphene levels ranging from 1280-5120 ppm to confirm the results and to extend the concentration range of the first study.

At 1280 ppm in the first and second studies, there were no deaths among rats and mean weight gains of both sexes were comparable to controls (see Table V-5). This is a NOAEL for mortality over a 6-week period. At 2560 ppm, two female rats died in the first study but mean weights of the survivors were not adversely affected. During the second study, one male and one female died at 2560 ppm. On the basis of these results, the low and high doses for the chronic studies were set at 1280 and 2560 ppm for males, and 640 and 1280 ppm for females.

In the same 1979 NCI subchronic study, mice were given feed containing 40-1280 ppm toxaphene in the diet. Four males and two females died at 640 ppm, and one male and one female given 320 ppm died. Mean weight gains of mice given 320 ppm were comparable with those of controls. On the basis of these results the low and high doses for the chronic studies were set at 160 and 320 ppm for males and females, respectively. The 160 ppm diet dose can be regarded as the NOAEL in mice with respect to mortality over a 6-week period.

Toxaphene aerosols in the form of dusts are more toxic to rats than in the form of mists. Mist concentrations as high as 500 mg toxaphene/m^3 caused no mortality in rats and rabbits over a 3-week period. However, no rats survived dust concentrations of 250 mg/m^3 for 1 week. Rats, dogs and guinea pigs were killed at 12 but not at 4 mg dust/m^3 over a 3-month period of exposure. Some surviving female rats exhibited slight focal hepatic cell necrosis (Boots Hercules Agrochemicals, Inc., n.d.).

Chronic Toxicity

Long-term exposures to low dietary levels of toxaphene are summarized in Table V-6. All studies note some form of liver pathology in rats at levels $\geq$100 ppm in the diet. At this same dose, cytoplasmic vacuolization similar to that seen following subchronic oral exposure was noted by Kennedy at al. (1973). Lehman (1952) also reported fatty degeneration of the liver in rats fed 100 ppm. With a 25 ppm diet, Fitzhugh and Nelson (1951) observed increased liver weight with minimal liver cell enlargement. Unpublished studies on rats, dogs and monkeys by Boots Hercules Agrochemicals, Inc. (n.d.) are in general agreement with the above published reports.

TABLE V-6

Chronic Toxicity of Toxaphene to Laboratory Mammals at Low Dietary Levels

Species (number of animals)	Duration of Feeding	Toxaphene Concentration (ppm diet)	Response	Reference
Rats, Sprague-Dawley (64M, 128F, weanling)	3 generations (42 weeks for F_0 and 39 weeks for F_1 and F_2)	25	No effect	Kennedy et al., 1973
		100	Liver pathology	
Rats	lifetime	25	No effect	Lehman, 1952
		100	Liver pathology	
Rats	lifetime	25	Liver pathology	Fitzhugh and Nelson, 1951
		100 (20M, 20F)	No effects	
		1000-1500 (20M, 20F for each dose)	Slight liver damage, CNS stimulation	
Dogs	2 years	5,10,20	No effect on organ weights, histology or clinical tests	Boots Hercules Agrochemicals, Inc., n.d.
	2 years	200 (1F, 1M)	Moderate liver degeneration	

TABLE V-6 (cont.)

Species	Duration of Feeding	Toxaphene Concentration (ppm diet)	Response	Reference
Dogs (6)	1360 days (3.7 years)	200* (4) (5 mg/kg/day)	Liver necrosis	Boots Hercules Agrochemicals, Inc., n.d.
	up to 1260 days	400* (2) (10 mg/kg/day)	One death after 33 days	
Bobwhite quail Colinus virginianus male	138-171 days	10.0	138 days 50% errors in behavioral tests	Kreitzer, 1980
		50.0	171 days-same as controls	
White Leghorn Chicks, female	up to 50 weeks	0.5	Occasional deformation of the cartilaginous region of the keel. Increased growth of cartilage.	Bush et al., 1977
		5.0	Sternal deformation and mild nephrosis at 5, 50 and 100.	
		50.0	Renal lesions at 50, 100	
		100.0	Decreased body weight	

*Administered in capsules containing toxaphene in corn oil

M - male; F = female

Kuz'minskaya and Ivanitskii (1979, 1981) showed that a single dose to rats, one-half of the LD_{50} of toxaphene (120 mg/kg bw), decreased the amount of adrenaline in adrenal glands and increased adrenaline and noradrenaline in the urine during the first 24 hours after administration of the pesticide. A chronic study was initiated using albino male rats (200-250 g) that were given daily doses of toxaphene (2.4 mg/kg bw) up to 6 months. The number of animals per group was not reported. Chronic administration altered catecholamine metabolism and breakdown. There were disturbances in catecholamine metabolism in toxaphene treated animals in the brain and the heart that may account for the clinically observed damage to the CNS and the myocardium.

The NCI (1979) conducted a chronic study with Osborne-Mendel rats and B6C3F1 mice to determine the possible carcinogenicity of toxaphene. Toxaphene was added to feed as described in the subchronic effects section. Fifty animals of each gender constituted a treatment group of rats or mice. Ten untreated animals of each gender were matched controls and data from 45 or 40 untreated animals from similar bioassays were pooled for statistical evaluations.

Groups of 50 mice/sex were administered toxaphene for 80 weeks and observed until sacrifice at 90-91 weeks (NCI, 1979) (Table V-7). Low-dose males and females received 160 ppm in the feed for 19 weeks followed by 80 ppm for 61 weeks; high-dose males and females, 320 ppm for 19 weeks, followed by 160 ppm for 61 weeks. TWA doses were 99 or 198 ppm in the diet for both males and females. Mean body weights attained by high-dose male mice were lower than those of matched controls, but weights of other exposure

TABLE V-7

National Cancer Institute Chronic Feeding Study[a]

Species	Duration of Feeding (weeks)	Toxaphene Concentration (ppm diet)	Time-Weighted Average Dose[b] (ppm diet)	Response
Mice, B6C3F1				
Males				
Low-dose	19 61	160 80	99	Mean body weights unaffected
High-dose	19 61	320 160	198	Mean body weights adversely affected; several animals died before week 19; dose-related decrease in survival
Females				
Low-dose	19 61	160 80	99	Mean body weights unaffected
High-dose	19 61	320 160	198	Mean body weights unaffected; several animals died before week 19; dose-related decrease in survival
Rats, Osborne-Mendel				
Males				
Low-dose	2 53 25	1280 640 320	556	Mean body weights unaffected
High-dose	2 53 25	2560 1280 640	1112	Hyperactivity (week 2); generalized body tremors (week 53); mean body weights unaffected; no dose-related decrease in survival

TABLE V-7 (cont.)

Species	Duration of Feeding (weeks)	Toxaphene Concentration (ppm diet)	Time-Weighted Average Dose[b] (ppm diet)	Response
Females				
Low-dose	55 25	640 320	540	Mean body weights adversely affected
High-dose	55 25	1280 640	1080	Mean body weights adversely affected; generalized body tremors (week 53); no dose-related decrease in survival

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[a] Source: NCI, 1979

[b] $\text{Time-weighted average dose} = \frac{\Sigma(\text{dose in ppm x no. of weeks at that dose})}{\Sigma(\text{no. of weeks receiving each dose})}$

groups were essentially unaffected by toxaphene. Several animals died in the high-dose groups before week 19 when doses were lowered. Following this, the exposed mice were generally comparable with controls in appearance and behavior during the first year of the study. During the second year, abdominal distention was observed in all dosed groups but predominantly in the high-dose males. Other clinical signs included alopecia, diarrhea, rough hair coats and dyspnea. From weeks 60-76 the low-dose males appeared hyperexcitable. After week 75 there were dose-related differences in survival. Because of the varied dose regimens, valid NOAEL data cannot be calculated.

Groups of 50 rats of each gender were administered toxaphene for 80 weeks (NCI, 1979) and then observed until survivors were sacrificed at 108-110 weeks (see Table V-7). Low-dose males were given 1280 ppm in the feed for 2 weeks, 640 ppm for 53 weeks and 320 ppm for 25 weeks, which was reported as a TWA of 556 ppm. High-dose males were administered 2560 ppm in the feed for 2 weeks, 1280 ppm for 53 weeks and 640 ppm for 25 weeks, or a TWA of 1112 ppm. Low-dose females received 640 ppm in the feed for 55 weeks and 320 ppm for 25 weeks, for a TWA of 540 ppm. High-dose females received 1280 ppm in the feed for 55 weeks, and 640 ppm for 25 weeks, for a TWA of 1080 ppm.

Mean body weights of the low- and high-dose female rats were lower than those of the matched controls throughout most of the bioassay study period, whereas weights of low- and high-dose males were essentially unaffected. During the first 16 weeks, dosed males were generally comparable with controls in appearance and behavior, with the exception of high-dose males that appeared hyperactive during week 2 when the doses for male rats were reduced. At week 53, the concentration of toxaphene in the feed was reduced because a majority of the high-dose males and females developed generalized body tremors. Dose-related decreases in survival rates were not observed. Clinical signs usually associated with aging were observed earlier in dosed rats than in controls and were as follows: alopecia, diarrhea, dyspnea, pale mucous membranes, rough hair coats, dermatitis, ataxia, leg paralysis, epistaxis, hematuria, abdominal distention and vaginal bleeding. Two females, one high-dose and one low-dose, had impaired equilibrium. Again, no valid NOAEL data is available for this study.

Bush et al. (1977) added graded levels of toxaphene in corn oil to the diets of female White Leghorn chicks from 1 day of age in order to achieve doses of 0, 0.5, 5, 50 and 100 ppm diet. Each treatment consisted of 90 randomly selected birds (30 birds in each of three replicates). Levels up to 100 ppm diet did not produce symptoms of toxicity throughout the 50-week study. Mortality was <5% in all groups. Body weights at 6 and 30 weeks were significantly decreased when the birds were fed 100 ppm. There were no significant treatment-related changes in heart, liver, gizzard or kidney weights at 4 or 8 weeks. Necropsy of 31-week-old birds fed 5, 50 and 100 ppm toxaphene in the diet revealed sternal deformation resembling osteomalacia. Occasional keel deformation involving the cartilaginous tissue as well as an apparent increase in growth of cartilage was found in birds fed 0.5 ppm toxaphene in the diet. Further studies were planned to determine whether the backbone of the chicken as well as other skeletal structures were affected by toxaphene. Histopathological examination of organs of 31-week-old birds showed mild nephrosis of the kidney in birds fed toxaphene at 5, 50 and 100 ppm. Other observations included cysts, occasional cellular casts in the renal tubules and accumulation of a brown granular pigment in the cytoplasm of some tubular epithelial cells.

Kreitzer (1980) determined whether behavioral effects in birds would be produced by toxaphene at levels below those that produced overt signs of intoxication. Adult male bobwhite quail _Colinus_ _virginianus_ were fed toxaphene dissolved in propylene glycol and blended into the feed at levels of 10 and 50 ppm. An equal amount of propylene glycol was added to the feed of controls. There were four controls and four birds per treatment level for each of two tests (with different pairs of patterns) that measured performance on nonspatial discrimination reversal tasks. Birds were initially exposed at 3 days of age for 138 and 171 days before tests 1 and 2, respectively. The treated birds in test 1 had 50% more errors than the controls ($p<0.02$). There were no significant differences in the performances of birds fed the two toxaphene levels. In test 2, after 171 days of exposure, treated birds performed as well as the controls, indicating an accomodation to the chemical.

Enzyme Effects

Toxaphene appears to induce the microsomal MFO system as evidence by the shortening of pentobarbital sleeping time at 50 mg toxaphene/kg bw (Schwabe and Wendling, 1967). A dose of 100 mg toxaphene/kg bw has been shown to decrease the barbiturate sleeping time by ~20% for up to 20 days after exposure (Ghazal, 1965). Toxaphene will also initiate MFO activity in whole liver homogenates from rats pretreated with toxaphene (Kinoshita et al., 1966). Effects in male rats were more pronounced than females. Toxaphene also appears to stimulate liver microsomal metabolism of estrone and testosterone. For example, an i.p. dose of 120 mg/kg bw to male rats caused a 484% increase in testosterone metabolism by 5 days after dosing and a 19% decrease in plasma testosterone, the latter level returning to control levels by the 15th day after dosing in spite of elevated microsomal enzyme activity by 532% (Peakall, 1976). For female rats pretreated at 25 mg/kg bw/day for 7 days, estrone metabolism increased by 155% (Welch et al., 1971). Since toxaphene is also known to bind to mammalian cytochrome P-450 to give a typical type I difference spectrum (Kulkarni et al., 1975), these effects may be attributed to induction of hepatic cytochrome P-450.

The toxicity of Toxicant B to mice is increased by a factor of 2-8 by administration of piperonyl butoxide that inhibits the cytochrome system (Saleh et al., 1977; Turner et al., 1977), which indicated to the authors that cytochrome P-450-mediated detoxification mechanisms were important to explain the mode of action of toxaphene toxicity (see Chapter VII). The rat liver microsomal system also mediates reductive dechlorination of Toxicant A and B (Khalifa et al., 1976; Saleh and Casida, 1978). NADPH and relatively anaerobic conditions were required. However, significant nonenzymic reaction in these systems may also occur as demonstrated by the ability of reduced hematin to cause reductive dechlorination (Saleh and Casida, 1978, 1979). The metabolites of these nonenzymic systems have already been identified in Chapter III, and reductive dechlorination and dehydrochlorination occur. Germinal and vicinal halogenated aliphatic organics are particularly susceptible.

Trottman and Desaiah (1980) fed male rats (Sprague-Dawley 175 g; 5-6/group) diets containing 0, 50, 100, 150 and 200 ppm toxaphene in the diet for 14 days causing increased liver weights at the 200 ppm dose and decreased thymus weight at the 150 and 200 ppm dose. In addition, enhancement occurred for liver hydroxylations of pentobarbital in a dose dependent manner, and for aniline and ethylmorphine-N-demethylase activity in a non-dose-dependent manner in the postmitochondrial supernatant fraction. Decreased sleeping time following pentobarbital administration also occurred, but was not dependent on dose. Hepatic microsomal protein content was increased 20-35% in comparison with control values. In the liver microsomal fraction, the activity of NADPH-cytochrome c-reductase was increased 20-50% at doses ≥150 ppm. Cytochrome P-450 levels were increased 15-27% at doses ≥50 ppm. There were no effects on the activity of NADPH-dehydrogenase or of the levels of cytochrome b_5. The binding of both aniline and hexobarbital to cytochrome P-450 was increased by 55-210% at all doses. All of these results show that specific functions of the MFO system of the liver, i.e., cytochrome P-450 and NADPH cytochrome c-reductase (the sites that are sensitive to aniline and hexobarbital interactions), are affected by toxaphene.

A toxaphene formulation was subfractionated on a silica gel column into 64 fractions of varying polarity (Pollock et al., 1983). The most highly toxic components to Sprague-Dawley rats were found in the nonpolar fractions. Young Sprague-Dawley rats (24 days; 70±6 g) were administered two fractions, one constituting 73% (the nonpolar fraction) and the other 27% (the polar fraction) at 25 mg/kg bw as well as unfractionated toxaphene at doses of 0, 5, 25 and 100 mg/kg bw by i.p. injection using olive oil carrier. Liver weights increased after administration of toxaphene doses of 25 mg/kg bw or more in a dose dependent manner, as also did the cytochrome P-450 level, aminopyrine demethylase activity and aldrin epoxidation. Microsomal protein was increased only at the 100 mg/kg dose. The protein content in the postmitochondrial supernatant was not affected by toxaphene. Both polar and nonpolar fractions caused the same results as toxaphene alone. The "no significant inductive effect" level for i.p. administered toxaphene is between the 5 and 25 mg/kg dosages.

Other enzyme systems are affected by toxaphene. One major system involves the metabolism of sugar to produce high energy compounds. At 330 µM, toxaphene inhibited the in vitro activity of mitochondrial succinoxidase in beef heart by 76% and the NADH-oxidase system by 96% (Pardini et al., 1971). At 100 µM, the active transport of glucose through isolated mice intestine was inhibited (Guthrie et al., 1974). Doses of 1.2 mg toxaphene/kg bw/day for 6 months or an acute 120 mg toxaphene/kg bw dose to rats altered lactate dehydrogenase (LDH) spectra in the liver and blood (Kuz'minskaya and Alekhina, 1976). In the liver, the acute dose caused a 45% inhibition of total LDH activity for 15 days postexposure. Increases in the isozymes, LDH-1, LDH-2 and LDH-3 were observed, LDH-4 only transiently decreasing and LDH-5 not at all. In the blood, the acute dose affected only LDH-1. Chronic exposures decreased total LDH activity in the blood (30%) and liver (45%). In the liver, LDH-3 increased after 3 months and LDH-5 decreased after 6 months. In the blood, LDH-1 and LDH-2 activities were depressed and LDH-5 activity elevated after 6 months. Depressed LDH activities were noted in the liver (17%), kidneys (18%) and serum (20%) of rats given 35 mg toxaphene/kg bw/day for 6 months (Gertig and Nowaczyk, 1975).

In the kidney, there was a 17% decrease in LDH-1. Decreases of 50% in both serum alkaline phosphatase and liver glutamate dehydrogenase also occurred after the 6-month exposure.

Peakall (1979), who gave male rats an acute 120 mg toxaphene/kg bw dose by capsule, showed that levels of pyruvate and lactic acid in blood plasma at 1, 5 and 15 days were not affected. This was so even when rats given 1.2 mg toxaphene/kg bw/day were observed at 1, 3 and 6 months. A steady-state level of toxaphene residues in the liver occurred after 1 month of chronic dosage; in the brain, this took 1-3 months. The author implied that the rats had to be stressed in some way to obtain the results of Kuz'minskaya and Alekhina (1976) and Gertig and Nowaczyk (1975). Peakall himself, however, gave no data on strain of rat, weight, gender or age.

Technical grade toxaphene was used in two studies by Srebocan et al. (1978, 1980a) on the enzymatic regulation of carbohydrate metabolism in poultry. In the 1978 study, groups of 1-day-old Nick chick cockerels (10 birds) were fed for 2 weeks on a formulated diet containing 0, 0.1, 0.5, 1, 5, 10, 50 or 100 ppm toxaphene (using sunflower oil to dissolve the pesticide and to mix it with the diet). Inhibition of enzymes of the gluconeogenic pathway in the liver was observed at 1 ppm. Enzymes involved in pyruvate metabolism were inhibited between 20 and 50% (pyruvate carboxylase, EC 6.4.1.1j and phosphoenolpyruvate carboxykinase, EC 4.1.1.32). There was not, however, a dose response. Fructose-1,6-diphosphatase (EC 3.1.3.11) and glucose-6-phosphatase (EC 3.1.3.9) activities, and glucose levels were not significantly affected at $p<0.05$. Pyruvate levels were increased by toxaphene ingestion. Based on studies with DDT, the authors suggested that the effects of a number of chlorinated pesticides were adrenocortically mediated. In the Srebocan et al. (1980a) study, the same experimental protocol was utilized except that toxaphene was given at 5 ppm in the diet separately or in combination with heat or starvation stress together with the appropriate controls. While toxaphene alone caused an accumulation of pyruvate (40%), heat stress alone also caused an accumulation of pyruvate (60%) and increased the activities of fructose-1,6-diphosphatase (15%) and glucose-6-phosphatase (17%); the combined heat and toxaphene treatment caused accumulation of pyruvate (17%) and increased the activity of glucose-6-phosphatase (17%). The activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and the level of glucose were not significantly affected by any of the treatments. For starvation stress/toxaphene experiments, all the toxaphene treatments (5 and 10 ppm diet) lowered the activities of all the above enzymes and increased the accumulation of pyruvate (70-80% excess). However control results were not consistent with those for the 5 ppm dose already quoted above. Starvation stress caused accumulations of pyruvate (20%) and enhanced fructose-1,6-diphosphatase activity (100%). The combination experiments led to inhibition of glucose-6-phosphatase (35-40%) and glucose depletion (35-40%) for the 5 and 10 ppm doses, and inhibition of pyruvate carboxylase (40%) and phosphoenolpyruvate carboxykinase (60%). Fructose-1,6-diphosphatase levels in the toxaphene and toxaphene/starvation experiments were not different from controls, whereas a large elevation occurred from starvation stress alone These results imply that stringent quality assurance of the environment and diets must be maintained during toxicological studies.

In an abstract, Desaiah et al. (1979) reported that male Sprague-Dawley rats fed 0, 25, 50 and 75 ppm toxaphene in the diet for 8 weeks showed marked dose-dependent increases in liver homogenate glucose-6-phosphatase and fructose-1,6-diphosphatase in the liver microsomal fraction, both being maximally stimulated by 70%. It was concluded that toxaphene affected the process of gluconeogenesis.

Alekhina and Kuz'minskaya (1980) determined LDH activity and changes in LDH isoenzymes in the liver and myocardium of "white" male rats (200-250 g), dosed perorally with 2.7 mg toxaphene/kg bw/day, percutaneously at 9.4 mg toxaphene/kg bw, and a combination group, all treatments being over 4 months. LDH activity in myocardium after 4 months increased 11-34% regardless of the mode of application, though the combination group gave the highest increase compared with the single treatments (16-18%). LDH activity increased (32%) in the liver only for the combination treatment. When provided orally, toxaphene caused increases in LDH-1 (65%) and a decrease in LDH-5 (27%); in the myocardium, decreases occurred in LDH-3 (35%) and in LDH-4 (70%). When provided percutaneously, increases in liver LDH-3 (64%) and LDH-4 (81%) were found as well as in myocardium (18 and 62%, respectively). LDH-5 was decreased (28%) only in the liver. For the combined treatment, the LDH-5 content in the liver remained decreased (25%), but LDH-3 and LDH-4 were increased by 24 and 140%, respectively. Although these changes in myocardium were postulated by the authors to be due to toxaphene residue deposition, this was not demonstrated directly.

Male albino Sprague-Dawley rats (300-350 g, 8/group) treated i.p. with a single 40 mg toxaphene/kg bw/day in vegetable oil carrier (0.5 mℓ) showed decreased (19%) plasma cholesterol only 60 days posttreatment, with no effect on plasma triglycerides, normalized liver weights, microsomal protein, or cytochrome P-450 (Ishikawa et al., 1978).

In another series of experiments, Kuz'minskaya et al. (1980) determined the oral and dermal toxicity of toxaphene in male rats separately and then investigated concomitant application of oral and dermal doses. Endpoints of toxicity included the activity of marker enzymes of mitochondria, microsomes and lysosomes in fractions of liver homogenates. Toxaphene applied _in vivo_ increased in 24 hours the activity of the liver mitochondrial enzymes succinic dehydrogenase 175% and cytochrome oxidase 1000%. Microsomal glucose-6-phosphatase activity was increased 41% and lysosomal acid phosphatase activity was increased 46%. The dose (or doses) that produced these responses was not clearly identified nor was the vehicle reported. The doses (mg/kg bw) toxaphene used in this study were as follows: oral, 67.5, 38.5 and 6.75; dermal, 235, 134 and 23.5. The greatest increase in enzyme activity was seen 24 hours after application.

Mehendale (1978) reported a decrease of biliary flow and decreased ^{14}C-imipramine excretion from the liver of male Sprague-Dawley rats dosed at 100 ppm toxaphene in the diet for 8 days. Trottman and Desaiah (1979) studied ATPase activities in the brain, kidney and liver of male ICR mice (30 g each) _in vivo_ and _in vitro_. For the _in vivo_ studies, toxaphene was provided at 0, 10, 25 and 50 mg/kg bw/day over 3 days by corn oil (0.3 mℓ) for 4-6 mice in each group. Enzyme activities were measured 36 hours after the last dosage. For *in vitro* studies, toxaphene at 7.5, 15.0 and 30 μM inhibited brain (52-56%) and kidney (42-46%) Na^+/K^+-ATPase activities, and the oligomycin-sensitive Mg^{2+}-activity in brain (53-61%), kidney (37-65%) and liver (20-62%). The inhibition of oligomycin-sensitive Mg^{2+}-ATPase activity was dose-dependent in the kidney and liver preparations. The oligomycin-insensitive Mg^{2+}-ATPase activity was also inhibited in brain (29-45%), kidney (47-50%) and liver (24-51%), the inhibition being almost dose-dependent for brain and liver. For *in vivo* studies, Na^+/K^+- and oligomycin-sensitive and insensitive Mg^{2+}-ATPase activities in toxaphene-treated mouse brain were not altered significantly. Kidney ATPases were significantly decreased (down to 42% for Na^+/K^+-ATPase activity; 35% for oligomycin-insensitive Mg^{2+}-ATPase) in a dose-dependent manner except for oligomycin-sensitive Mg^{2+}-ATPase. A dose-dependent decrease in the oligomycin-sensitive Mg^{2+}-ATPase activity (down to 35%) was found in the liver, but, in contrast with the kidney, all doses increased the oligomycin-insensitive component (over 60%). The differences between the *in vivo* and *in vitro* results for the brain may arise from the fact that toxaphene may be metabolized extensively in *in vivo* studies before it reaches the liver (perhaps by reduced hematin or another nonenzymatic process), kidney or brain. Rapid metabolism may also account for the effects on ATPases in kidney and liver. The crucial presence or absence of toxaphene after addition in the *in vitro* or *in vivo* studies was not directly measured. The Na^+/K^+-ATPase is postulated to be involved in active ion transport; the oligomycin-sensitive Mg^{2+}-ATPase in mitochondria is believed to be involved in oxidative phosphorylation. The interference of toxaphene with the production of ATP may cause an impairment of transport processes in the liver and kidney, and may be related to impairment of biliary excretion.

In another study (Trottman and Desaiah, 1983), adult male Sprague-Dawley rats (175-200 g; 6/group) were fed 0, 50, 100, 150 and 200 ppm toxaphene in powdered chow for 8 weeks. Toxaphene inhibited Na^+/K^+- and Mg^{2+}-ATPases of synaptosomes *in vitro* in a dose-dependent manner. The IC_{50} values were about 30 μM toxaphene for Na^+/K^+-ATPase and 15 μM toxaphene for the Mg^{2+}-ATPase. Fifty percent inhibition of ouabain and dopamine binding occurred around 150 and 200 μM toxaphene, respectively. For *in vivo* studies, no dose responses were observed for decrease in enzyme activities (30-40% decreases for doses ≥100 ppm) even though all ATPase activities were decreased. In fact, there were no significant changes for *in vivo* studies involving ouabain and dopamine binding to synaptosomes. Toxaphene was postulated by the authors to have metabolized rapidly *in vivo* and not reached the brain to explain the difference in the *in vivo* and *in vitro* results. Na^+/K^+-ATPase has been shown to be a receptor of ouabain and perhaps dopamine. Toxaphene itself interferes *in vitro* but not *in vivo*, and caution therefore must be exercised in the use of *in vitro* tests in place of *in vivo* tests. Fattah and Crowder (1980) also tested the effects of toxaphene *in vivo* and *in vitro* on the plasma membrane ATPase of kidney, brain and liver from mice. Male Swiss Webster mice (35 per treatment and control groups) were administered by oral gavage an LD_{50} (112 mg/kg bw) dose of toxaphene in corn oil, or corn oil alone. Kidney ATPases were more sensitive to toxaphene than the liver and brain enzymes. The Na^+/K^+-ATPase was inhibited significantly only in the kidney, and the Mg^{2+}-ATPase was inhibited significantly in all three tissues. Twenty mice were used to study the effects of *in vitro* toxaphene exposure of tissues. Each tissue was tested with four concentrations of toxaphene, 10^{-4}, 10^{-5}, 10^{-6} and

10^{-7} M, and two controls. Three samples for each treatment were run in each of the three tissues. As observed following in vivo exposure, toxaphene significantly inhibited the Na^+/K^+-ATPase activity only in the kidney homogenates, in this case with a dose effect. Mg^{2+}-ATPase was significantly reduced in all tissues; in the liver homogenates only 10^{-4} M toxaphene significantly inhibited activity.

Parvu et al. (1980) reported in an abstract that toxaphene given orally to white Wistar rats at 4 mg/kg bw/day for 30 days caused lipid accumulation in the liver, depletion of free fatty acids, and an increase of liver cholesterol.

Toxaphene may also affect Vitamin A storage. A concentration of 100 ppm toxaphene in the diet for 72 days to 28-day female weanling rats (Phillips and Hatina, 1972) was followed by mating with untreated males. At 20 days postpartum, liver Vitamin A levels relative to controls were depressed by 17% ($p<0.01$) in newborn rats, but not in dams.

Alterations in clinical chemistry have also been seen in subchronic oral toxaphene exposures. Mice with no clinical signs of intoxication evidenced consistent increases in serum acid phosphatase, glutamic pyruvic transaminase and gamma-glutamyl transpeptidase activities, along with increased neutrophil counts and changes in urine protein (Bäumler, 1975). At a much lower daily dose, rats had only a transient increase in serum alkaline phosphatase during the fifth month of ingestion and showed no variation in urine hippuric acid (Grebenyuk, 1970). Increases in all of the above enzyme activities are consistent with the mild liver pathology associated with subchronic toxaphene exposure.

Teratogenicity and Reproductive Effects

Mammals. The multigeneration reproductive effects of toxaphene and two other pesticides were investigated by Kennedy et al. (1973). Two control groups and two test groups of Sprague-Dawley albino rats consisted of 8 male and 16 female animals each. The treated groups started receiving 25 or 100 ppm toxaphene in the diet (adjusted to contain 2% corn oil) added to commercial chow at 28 days of age. F_0 parental animals were exposed to toxaphene for 42 weeks and then sacrificed. F_1 and F_2 parents were exposed for 39 weeks and then sacrificed. Animals were first mated at 100 days of age. First litters were reduced to 10/litter on postpartum day 5 and retained for 21 days until weaning. After a 10-day rest, the parental animals were mated again to obtain second litters. At weaning of the second litters, 8 males and 16 females were randomly selected from each group as parental animals for the next generation. This procedure was continued through three successive 2-litter generations. Toxaphene had no effect on litter size, pup survival or weanling body weights. Parental animals at 100 ppm showed liver changes consisting of slight cytoplasmic fatty vacuolization in 63% of animals examined. This change was not observed at 25 ppm and was not accompanied by adverse effects on growth, mortality, organ weights (liver, kidney, spleen, gonads, heart, brain, adrenal glands, thyroid gland) or among the corresponding organ-to-body weight ratios or reproductive capacity. No differences were observed among test and control groups of F_2 parental animals with regard to total, free and esterified cholesterol concentrations, hematologic parameters, urine analysis and other clinical chemical findings. Tumor incidence was not affected and there was no evidence of teratogenicity among test progeny. The no-effect level was judged to be 25 ppm in the diet for toxaphene (1.25 mg/kg by assuming food intake equal to 5% of body weight).

Chernoff and Carver (1976) used rather high doses of toxaphene to study fetal toxicity of the pesticide in rats. CD rats were administered toxaphene in corn oil (0.1 mℓ) by gastric intubation on days 7 through 16 of gestation at doses of 15, 25 or 35 mg/kg bw. The number of rats per group were as follows: controls, 33 rats; 15 and 25 mg/kg, 39 rats/group; 35 mg/kg, 16 rats. At 35 mg toxaphene/kg, toxicity was evidenced by 31% maternal mortality. There was also a dose-related reduction in weight gain of dams ($p<0.001$) at 15 and 25 mg/kg/day and fetal weight ($p<0.001$) at 25 mg toxaphene/kg/day. Even though there was significant maternal toxicity in all treated groups, there were no dose related changes in fetal mortality or in the occurrence of anomalies.

The rat fetus was shown to contain a small amount of ^{14}C-toxaphene when assayed 5 days after an oral dose of 2.6 or 6.5 mg/kg maternal body weight on day 15 of gestation (Pollock and Hillstrand, 1982) Fetal tissues contained 28 μg/kg wet weight; in comparison, maternal fat contained 7.48 mg/kg tissue.

Mixed-strain white rats and golden hamsters were used by Martson and Shepel'skaya (1980b) to evaluate the embryotoxicity of toxaphene. Rats were given 4 mg toxaphene/kg bw/day by gastric intubation during the period of organogenesis (days 6-15) and during the entire pregnancy (1-31 days) and to hamsters on days 7-11 and 1-15 of gestation. In rats, no adverse effects were noted on development, fetal weight, ratio of males to females, or the number of fetal deaths. In hamsters, the only toxaphene-related effect was reported as an increase in an unidentified developmental anomaly.

Badaeva (1979, 1981) examined the effects of toxaphene on cholinesterase activity in cardiac neural structures of dams and future offspring on days 14 and 20 of gestation. A group of 12 pregnant animals was perorally administered daily doses of 12 mg toxaphene/kg bw. It was found that the number of dead embryos was higher when toxaphene was administered throughout gestation than when administered between the 6th and 14th day of gestation. When given throughout gestation, the amount of toxaphene found in the maternal heart was higher on the 20th day of gestation (14 μg/g), uterus (9.7 μg/g), brain and spinal cord (4.3 μg/g) than on the 14th day. The number of pups per litter (6-8) was less in treated groups than in control groups (10-11); birth weight of pups was depressed and increased mortality was noted in the first week postpartum. In treated embryos, cholinesterase activity along the myocardial vessels and cells of the atrioventricular node was almost absent at 14 days of gestation; at 20 days, there was a delay in differentiation of fetal cardiac neural elements and a continued depression of cholinesterase activity. On the 20th day, 50-60% of the cells of the cardiac ganglion of pregnant treated animals exhibited decreased cytoplasmic cholinesterase activity.

Toxaphene was one of five compounds administered to pregnant 90-day-old CD rats during the period of organogenesis (7-16 days of gestation), and the effects on organ differentiation were determined in day 21 fetuses (Kavlock et al., 1982). Two dose levels of toxaphene in corn oil, 12.5 and 25 mg/kg bw/day, administered perorally to a group of five animals, had no effect on the average number of implants, fetal mortality or fetal weight. No soft tissue abnormalities were observed. There was no statistical difference between the following parameters in treated and untreated rats: brain weight, total DNA and total protein; lung weight and primary surfactant materials; liver weight and total glycogen; and kidney weight. There was a statistically significant decrease in alkaline phosphatase activity of the kidney at 25 mg toxaphene/kg bw and in total protein in the kidney at both doses.

Behavioral effects of low levels of toxaphene, and its toxic components, Toxicants A and B, were studied in juvenile albino rats exposed perinatally (Olson et al., 1980). Toxaphene, or Toxicant A or B, was given daily in the feed, starting on day 5 of gestation. Pups were exposed to dietary levels after weaning until the study was terminated at 3 months postpartum: toxaphene 50 µg/kg bw, Toxicants A and B, 2.0 µg/kg bw. Because sufficient supplies of Toxicant A could not be procured, Toxicant B was substituted for Toxicant A when supplies were depleted (rats were 40 days old). Offspring from 12 mothers made up three treatment groups of 16 each and one control group of 15. Behavioral testing began when the offspring were 7 days old. Two different behavioral test periods were studied: early development with pups being tested on postnatal days 7 through 17 (swimming and righting reflex); motivational, learning and retention tests on days 70 through 90 (symmetrical maze). In their early development, treated animals showed retarded maturation based on their performance in overall swimming ability in comparison with control animal performance ($p<0.0001$ on day 10). By day 16 all groups displayed normal swimming ability. Inferior swimming ability may reflect an ability of toxaphene compounds to affect early functional and behavioral development of the CNS in the immature rat. Toxaphene-fed animals constituted the only group to exhibit overall retarded righting ability in tests conducted from days 7 through 17. On day 15 the order of decreasing superiority was as follows: controls, Toxicant A, Toxicant B and toxaphene ($p<0.0001$) with each group being significantly superior to the one following. Inability of the immature rat to metabolize the simple mixture may account for the increased toxicity of toxaphene. The test of motivation revealed no significant differences between any of the groups. In the maze retention test, Toxicant A animals had no difficulty in learning the test problems but were inferior to the other groups in retaining that knowledge. Behavioral changes were observed at dosages far lower than those used in any previously reported study.

In another behavioral study, Crowder et al. (1980) also reported a significant difference in the length of time for treated animals to become positive in the righting reflex. In the Crowder et al. (1980) study, three pregnant rats received daily oral doses of 6 mg/kg bw in 0.1 mℓ corn oil from day 7 of gestation until parturition. Testing methods included a simple two-choice maze, motor skills, an open field test and other behavioral tests.

Investigators in Russia found no effect on behavioral responses of 1-month-old pups exposed during days 6-15 of gestation (Martson and Shepel'skaya, 1980a). The dose was reported as the 0.1 LD_{50} given by gastric intubation to six pregnant animals; however, the LD_{50} for the mixed strain rats was not given. In this same study, 6 male and 6 female rats received 4 mg toxaphene/kg bw by gastric intubation daily for 10 weeks before mating. Males and females receiving toxaphene were bred with untreated rats. At the age of 2 months, offspring were monitored for behavioral response. There were no toxic effects observed in the offspring as judged by locomotion, grooming and rearing activities. The authors concluded that toxaphene did not have major gonadotoxic effects. There was, however, an increase in the number of sperm with abnormal morphology (Martson and Shepel'skaya, 1980b).

DiPasquale (1977) examined the effects of toxaphene on fetal guinea pig development. In this study, toxaphene was administered orally to pregnant females at a dose of 15 mg/kg bw from day 21 to day 35 of gestation. No effects were noted on the anatomical development of the fetus. There was some fetotoxicity as shown by adverse effects on collagen-related structures of the fetus. This was attributed to a functional deficiency of vitamin C related to MFO induction. Maternal guinea pigs showed a slight loss of body weight, but no effects attributable to toxaphene exposure were seen on maternal liver weight or mortality.

CD-1 albino mice were administered toxaphene in corn oil by gastric intubation during the period of organogenesis, days 7 through 16 of gestation (Chernoff and Carver, 1976). The number of mice per group and daily doses of toxaphene were as follows: 75 mice, 0 toxaphene; 26 mice, 15 mg/kg bw; 45 mice, 25 mg/kg bw; 90 mice, 35 mg/kg bw. Mice were killed on day 18 of gestation and fetuses weighed and prepared for soft tissue or skeletal analysis. In the 35 mg/kg group there was 8% maternal mortality. There was a dose-related ($p<0.01$) reduction in the average maternal weight gain and a dose-related increase in the liver-to-body weight ratio. There were no significant dose-related responses in fetal mortality, fetal weight, number of caudal or sternal ossification centers, or incidence of supernumerary ribs. Five litters from dams receiving the high toxaphene dose had one or more fetuses with encephaloceles; none were observed in controls or other treated groups.

Keplinger et al. (1970) conducted a 5-6 generation study of five pesticides including toxaphene and combinations of pesticides using Swiss white mice. Offspring were exposed _in utero_, by the mother's milk, and in the diet after pups were weaned. Toxaphene (25 ppm in the diet) was fed to mice during the entire study including mating, gestation and lactation. Animals not used for subsequent breeding were sacrified for chemical analyses and histology. Eighteen 120-day-old mice, 4 males and 14 females, made up each group. For each successive generation, parental groups were remated to produce second litters and the next parental groups were selected from second litters when 120 days of age. Little or no adverse effect was noted through five generations of mice fed 25 ppm toxaphene in the diet including the following parameters for each generation: litter size, embryonic death, survival and body weight. There were no statistically significant differences between several indices calculated by the investigators including viability, lactation, survival and reproduction. Histologically there was evidence of fatty changes in the liver, especially in the central zone. There was little or no damage to the brain.

Twenty-eight compounds of known teratogenic potential were assayed by an _in vivo_ teratology screening procedure (Chernoff and Kavlock, 1982). Toxaphene in corn oil was administered by oral gavage (75 mg/kg bw) to 25 gravid CD-1 mice on days 8-12 gestation, the period of organogenesis. Two mice died before term and only 11 were pregnant. Dams were allowed to give birth, and litter size and weight on postpartum days 1 and 3 were compared with concurrent controls. Results indicated significantly reduced maternal weight change and reduced fetal weights on postpartum day 1. There was no difference in the body weights of treated versus control pups on postpartum day 3.

Allen et al. (1983) studied the effect of toxaphene on immune responses of offspring of mice exposed orally 3 weeks before breeding, and during gestation and lactation. Toxaphene in acetone was mixed into ground rat pellets to attain a concentration of 10, 100 or 200 ppm. Transplacental/lactation exposure to toxaphene produced some degree of immunosuppression in the pups, even though overt toxicity of the insecticide was not apparent at the lower dose. At 10 ppm there was a significant depression of macrophage phagocytosis ($p<0.05$) but no effect on hypersensitivity responses and antibody titers to bovine serum albumin. Relative degree of immunosuppression was greatest in macrophages followed by humoral immunity; cell-mediated immunity was least affected.

Birds. The effects of toxaphene have been examined in a number of bird species. White leghorn female chicks, 1 day of age, were fed diets containing 0, 0.5, 5, 50 and 100 ppm toxaphene (Bush et al., 1977). Each treatment group contained 90 randomly selected hens that were bred at 23 weeks of age. Even at 100 ppm, toxaphene did not significantly alter egg production, hatchability or fertility. Arscott et al. (1976) added 0, 10 and 100 ppm toxaphene to the feed of white leghorn layers (48/group) for 24 weeks. Except for a slight decrease in egg production, no adverse effects were observed on fertility, hatchability and survival of progeny.

Three groups of 15 pairs of 1-year-old black ducks (Anas rubripes) were fed ad libitum wih commercial duck breeder mash coated with 0, 10 or 50 ppm toxaphene beginning 90 days prior to laying (Mehrle et al. 1979). Toxaphene was dissolved in propylene glycol equivalent to 1% of the total diet. Reproductive success of all birds was followed throughout the breeding season. Toxaphene did not affect reproduction or survival. Ducklings were fed starter mash containing the same concentration of toxaphene as the diet the parents received. Duckling growth, as indicated by weight, was decreased significantly 5 and 14 days after hatching in the group fed 50 ppm, and was increased significantly at 28 and 42 days after hatching in the group fed a diet containing 10 ppm toxaphene in the diet. Duckling weight was not altered by toxaphene in measurements made more than 42 days after hatching. Backbone development was impaired by toxaphene within 14 days after hatching and collagen was decreased significantly in the cervical vertebrae of ducklings fed 50 ppm. In contrast to the effects on vertebrae, toxaphene exposure did not alter tibia development.

Forty-five pairs of 1-year-old black ducks (Anas rubripes) were placed on a diet of duck breeder mash containing 0, 10 or 50 ppm toxaphene (Heinz and Finley, 1978). Toxaphene was dissolved in propylene glycol before incorporation into the feed at 1 part/99 parts feed. About 3 months later, hens began to lay eggs and the eggs were incubated. Ducklings were brooded by hens and ate a diet of duck starter mash containing the same concentrations of toxaphene as those fed the hens. At 5 days of age, ducklings were tested for avoidance behavior. There were no significant differences in avoidance behavior between control ducklings and those treated with 10 or 50 ppm toxaphene.

In a similar study conducted over a 19-month period, which included two breeding seasons, three sets of 15 pairs of black ducks (Anas rubripes) were given 0, 10 or 50 ppm toxaphene in dry mash (Haseltine et al., 1980). Toxaphene was dissolved in propylene glycol before incorporation into the feed at 1% of feed by weight. Adult survival was not affected, but the weights of treated males were depressed during the summer months. At the end of the second breeding season females ingesting 10 or 50 ppm had larger livers than controls. Drakes fed 50 ppm in the diet exhibited elevated brain weights that were not apparent in 50 ppm females. In both years of the study, breeding pairs fed 50 ppm toxaphene in the diet appeared hyperactive but caging facilities did not allow for testing of this possibility. Egg production, fertility, hatchability, eggshell thickness, growth and survival of young did not vary significantly with toxaphene ingestion in either breeding season. The mean number of days required to complete a clutch was lower in birds fed toxaphene than in controls. Clutches of hens fed 50 ppm toxaphene showed improved hatching success in the second year of the study compared with the first year.

Toxaphene was injected at levels of 0.1, 0.5, 1.0, 5.0 and 10 mg/kg egg weight into groups of 20 fertile Nick chick eggs on day 0 of their incubation until day 14 (Srebocan et al., 1980b). The injection was made in the air sac and contained 0.1 mℓ of the toxaphene solution in sunflower oil. The following assays were carried out on nine or more embryo livers per treatment: pyruvate, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase, glucose-6-phosphatase and glucose. The effect on carbohydrate metabolism was similar in all groups. Increased pyruvate concentration, decreased glucose concentration and decreased enzyme activities, particularly in the embryos injected with the higher doses, indicated that toxaphene significantly inhibited the gluconeogenic pathway in the embryonic liver. Lower reproductive success might also cause embryonic death or a lowered vitality of hatched chicks.

Hoffman and Eastin (1982) determined the effects of externally treating mallard (Anas platyrhychos) eggs with formulations and concentrations of toxaphene similar to those in field applications. Toxaphene, in aqueous emulsion applied on day 3 after the eggs were placed in an incubator, resulted in significant mortality ($p<0.01$) even at one-half the field level of application (12.5 lb/acre at 100 gal/acre); the LC_{50} was 108 lb/acre (121 kg/hectare). At concentrations greater than the LC_{50} there were abnormal survivors that exhibited dislocation of joints. Treatment on day 8 at 5 times the field level resulted in embryotoxic effects including 53% mortality, a reduction in growth, and an increased incidence of abnormal survivors (18%), some with joint defects. When toxaphene was applied in an oil vehicle either at day 3 or day 8, the LC_{50}s were greater than for the concentrated formulation (66 lb/A). Treatment on day 8 resulted in growth reduction ($p<0.05$). Higher concentrations resulted in brain, bill and joint defects. Exposures up to 5 times the field level did not grossly affect cervical vertebrae as revealed by alizarin red staining.

Mutagenicity

The National Toxicology Program (NTP, 1983) reported toxaphene to be mutagenic when assayed by the Salmonella/microsomal reverse mutation assay. Hill (1977), summarizing tests done by Litton Bionetics for Hercules, Inc., indicated that toxaphene was directly mutagenic only for Salmonella typhimurium strains TA98 (which detects frameshift mutagens) and TA100 (nonspecific). By contrast, a "high temperature" toxaphene (high boiling components) was mutagenic only when rat liver preparations (S9) were incorporated in the assay to provide for metabolism of the compounds to biologically active forms. Hooper et al. (1979) used Salmonella mutagenicity assays to identify toxaphene components of potential carcinogenic hazard. TA100 proved to be the most sensitive indicator of toxaphene mutagenicity and it was determined that mutagenicity resided in the polar fraction. Mutagenic activity was reduced by 50% upon addition of rat or mouse S9 to the assay plates. A major toxic component of toxaphene, Toxicant B, was not mutagenic for any of five Salmonella strains encompassing three DNA sites. Treatment of toxaphene with ethanolic KOH in molar ratios of 1:1 or 1:10 for 24 hours at 25°C resulted in dechlorination of the major gem-dichloro toxic components. This treatment did not affect the S9-independent mutagenicity detected by TA100. Recrystallization from isopropanol, resulting in a less polar mixture, decreased mutagenic activity. Toxaphene applied to a silicic acid column was successively eluted with hexane and methanol. The methanol fraction was essentially free of those components known to be toxic to animals; however, this fraction was far more mutagenic than the hexane fraction (2640 revertants/mg vs. 200). Further separation of the highly mutagenic component(s) was unsuccessful. Hooper et al. (1979) also tested samples of 10 commercial preparations produced by Hercules, Inc. from 1949 to 1975. While these materials were similar in toxicity and in retention as determined by open tubular-column gas chromatography, they varied in mutagenic activity from 310 revertants/mg (mixture manufactured in 1954) to 1270 revertants/mg (mixture made in 1963).

Griffin and Hill (1978) described use of an *in vitro* DNA breakage assay utilizing purified covalent closed circular DNA molecules of plasmid ColE1. Breakage rates were determined after analysis by alkaline sucrose gradient centrifugation. Breakage rates significantly greater than control were obtained with three mutagenic alkylating agents and 4/11 pesticides tested (dexon, dichlorvos, malathion and methyl parathion). Toxaphene incubated at 0.1 mg/mℓ in hexane with plasmid DNA for an unspecified amount of time caused no increase in breaks.

Epstein et al. (1972) used a modified dominant lethal assay in mice to evaluate the mutagenic potential of a variety of chemical agents including toxaphene. In this study, four groups of male ICR/Ha Swiss mice were given toxaphene either intraperitoneally (single doses of 36 or 180 mg/kg) or orally (five doses of 8 or 16 mg/kg/dose). After dosing, the treated males were mated to three untreated females/week over an 8-week period. Females were dissected 13 days following the midweek of their caging with the males. Based on measurements of early fetal deaths per pregnancy and the percentage of females with early fetal deaths, there was no significant difference between control and toxaphene treated groups. Thus, in this strain of mice toxaphene apparently does not produce chromosomal abnormalities in sperm that preclude zygote development.

Sobti et al. (1983) assayed organochlorine pesticides for their ability to cause sister chromatid exchanges (SCE) in a human lymphoid cell line, LAZ-007, of B cell origin. Significant increases in numbers of SCE were observed with toxaphene concentrations of 10^{-5} and 10^{-4} M. SCE frequency, however, did not reach the level of 2 times control frequency that has been recommended as the criterion for a positive response (Latt et al., 1981). Samosh (1974) reported increased frequency of chromosomal aberrations in lymphocyte cultures obtained from eight women occupationally exposed in a spraying operation to 2 kg/ha (13.1% in exposed vs. 1.6% in control). Aberrations consisted of acentric fragments and chromatid exchanges. This is by contrast to a U.S. EPA (1978) study that found no significant differences in rates of chromosomal aberrations in leukocytes between groups of individuals occupationally exposed to toxaphene and unexposed groups.

Carcinogenicity

The most definitive studies of toxaphene carcinogenicity were performed by Tracor Jitco Co. under contract from the National Cancer Institute (NCI, 1979) in spite of the fact they were not conducted in accordance with NCI guidelines (control groups contained only 10 animals each; pair-feeding was also not done). In this study both genders of Osborne-Mendel rats and B6C3F1 mice of age 35 days were used. Each experimental group contained 50 animals of each gender, and the pesticide was added to the diet as an acetone solution, 2% corn oil also being added as a dust suppressant. The high and low male rat diets initially contained 2560 and 1280 ppm toxaphene, respectively, and the corresponding diets for females contained 1280 and 640 ppm, respectively. In the case of mice the corresponding high and low dose figures were 320 and 160 ppm diet for both sexes. Owing to overt toxicity these concentrations were later lowered. In male rats the high dose was lowered to 1280 ppm at 2 weeks, and to 640 ppm at 55 weeks after initiation of the study. The low dose was similarly lowered to 640 ppm diet after 2 weeks and 320 ppm 55 weeks after feeding had begun. In the case of females, both the doses were halved after 55 weeks, and for both sexes pesticide treatment was discontinued after 80 weeks. Subsequently the animals were fed control diets without corn oil for 20 weeks and then control diets for an additional 8 weeks. In male and female mice both doses were halved 19 weeks after treatment was initiated; toxaphene treatment discontinued after 80 weeks, and animals were fed control diets without corn oil for 7 weeks then diets with 2% corn oil for an additional 3-4 weeks.

Animals that died during the study, and also all animals surviving at the termination of the study, were submitted to pathologic evaluation of major tissues, major organs and all gross lesions. In the male rats, 90% of the high dose, 94% of the low dose and all of the control group lived until at least week 52 of the study. In females 96% of the high dose, 92% of the low dose and all 10 control animals survived beyond the 52nd week. Although none of the tumors observed in treated animals were uncommon for the animal strain used, certain tumors and hyperplastic lesions were present with higher incidence in the treated animals. These included thyroid follicular cell adenomas and carcinomas (7/41 low-dose, 9/35 high-dose and 1/7 control males; 1/43 low-dose, 7/42 high-dose and 0/6 control females) and hyperplasias (3/41 low-dose, 3/35 high-dose and 0/7 control males; 5/43 low-dose, 3/42 high-dose and 0/6 control females). The thyroid follicular-cell hyperplasias were observed only in the treated animals and were at relatively low incidences. Therefore it could not be concluded that increased incidences of neoplasms or proliferative lesions were a result of toxaphene treatment. These authors regarded adenomas as neoplastic lesions; however, this is controversial. Taking thyroid follicular cell adenomas and carcinomas together, a statistically significant increase was found for the high-dose group compared with the matched controls for both male and female rats (Tables V-8 and V-9, respectively). In comparison with values from historical controls from the same laboratory, statistical significance was also found for the above lesions. In the female rats there was also a statistically significant increase in the cumulative incidence of tumors of the pituitary (chromophobe adenomas, chromophobe carcinomas or adenomas) in the high dose compared with the control group, although it is warranted only to refer to the carcinomas as neoplastic lesions. Due to the high spontaneous incidences seen in controls, NCI does not conclude that an association exists between toxaphene administration and an increased incidence of pituitary tumors. From the above study the authors concluded that toxaphene administration was associated with an increase in thyroid tumor incidence.

Following an independent examination of histological preparations, Reuber (1979) came to similar qualitative conclusions, reporting 10, 15 and 28% thyroid adenoma and 6, 18 and 18% thyroid carcinoma incidence in control male rats or in groups ingesting the low or high dose of toxaphene, respectively. In females, the corresponding figures were 6, 18 and 19% for adenoma, and 10, 9 and 20% for carcinoma incidence. In addition, Reuber (1979) claimed increases in benign and malignant tumor incidence in endocrine organs, reproductive system and mammary glands in toxaphene-treated rats. Until differences between Reuber's criteria and those of others are resolved, however, it will be difficult to draw conclusions from his findings.

TABLE V-8

Incidence of Tumors in Male Rats Fed Toxaphene[a]

Dose (ppm diet)	Number of Animals Initial	Number of Animals Examined	Skin	Hemato-poietic	Kidney	Pituitary	Adrenal	Thyroid	Prostate	Liver[b]	Musculo-skeletal	Meso-thelioma	Nervous System	Total[c]
							MALIGNANT							
0 Matched	10	9	0/10	0/10	0/9	0/7	0/9	0/7	0/9	1/9(11%)	0/10	0/10	0/9	0
Low	50	47	2/50(4%)^{d}	0/50(0%)	1/45(2%)	0/42	1/41(2%)	1/41(2%)	0/37	6/44(14%)	0/50	0/50	0/50	7
High	50	45	1/45(2%)	2/45(4%)	1/45(4%)	1/31(3%)	0/37	2/35(6%)	1/35(3%)	4/45(9%)	1/45(2%)	0/45	0/45	10
							BENIGN							
0 Matched	10	9	0/10	0/9	0/9	1/7(14%)^{e} 2/7(29%)^{g}	2/9(22%) 2/9(22%) 1/9(11%)	1/7(14%)^{f}	0/9	0/9	0/10	0/10	0/9	7
Low	50	47	2/50(4%)	3/45(7%)	2/45(4%)	1/42(2%)^{e} 12/42(29%)^{g}	4/41(10%)^{h}	7/41(17%)^{f} 1/41(2%)^{i} 1/26(4%)^{j}	0/37	0/44	0/50	0/50	2/50(4%)	27
High	50	45	1/45(2%)	3/42(7%)	2/45(4%)	4/31(13%)^{g}	3/37(8%)^{h} 1/37(3%)^{l}	7/35(20%)^{f,k}	0/35	0/45	1/45(2%)	1/45(2%)	4/45(2%)	20

[a] Source: NCI, 1979

[b] Liver = neoplastic nodules

[c] Total = total animals with tumors

[d] $\frac{\text{Number of animals bearing tumor stated}}{\text{Number of animals with tissue stated examined microscopically}}$

[e] Adenoma

[f] Follicular cell adenoma

[g] Chromophobe adenoma

[h] Cortical adenoma

[i] C-cell adenoma

[j] Adenoma of parathyroid

[k] Adenoma + carcinoma incidence significant (Fisher exact text, p=0.008) compared with matched controls

[l] Pheochromocytoma

TABLE V-9

Incidence of Tumors in Female Rats Fed Toxaphene[a]

Dose (ppm diet)	Number of Animals Initial	Number of Animals Examined	Skin	Lung	Liver	Pituitary	Adrenal	Thyroid	Mammary	Uterus	Ovary	Kidney	Total[b]
							MALIGNANT						
0 Matched	10	10	0/10	0/9	1/10(10%)	0/8	0/8	1/6(17%)	0/10	0/9	0/8	0/8	1
Low	50	49	1/50(2%)[c]	0/46	4/42(10%)[d] 1/42(2%)[e]	0/41	0/44	0/43	1/50(2%)	1/41(2%)	0/40	0/49	4
High	50	49	3/49(6%)	1/48(2%)	4/40(10%)	1/39(3%)[f]	2/43(5%)	0/42	3/49(6%)	0/45	1/36(3%)	0/48	10
							BENIGN						
0 Matched	10	10	0/10	0/9	0/10	1/8(13%)[g] 2/8(25%)[i]	0/8	0/6	1/10(10%)		0/8	0/10	4
Low	50	49	0/50	0/46	1/46(2%)[m]	15/49(37%)[i]	3/44(7%)[j]	1/43(2%)	2/50(4%)[g] 1/50(2%)[k] 10/50(20%)[l]	9/50[o]	1/50(2%)	0/49	28
High	50	49	1/49(2%)	0/48	1/40(3%)[h]	18/39(46%)[i] 4/39(10%)[f,g]	4/43(9%)[j]	7/42(17%)[n]	1/49(2%)[g] 2/49(4%)[k] 10/49(20%)[l]	1/45(2%)[j] 5/45(11%)[o]	0/36	1/48(2%)	36

[a] Source: NCI, 1979

[b] Total = total animals with tumors

[c] $\frac{\text{Number of animals bearing tumor stated}}{\text{Number of animals with tissue stated examined microscopically}}$

[d] Neoplastic nodule in the liver of low dose

[e] Hepatocellular carcinoma in the liver of low dose

[f] Total tumor incidence of tumors in pituitary (p=0.012, Cochran-Armitage test) compared with pooled controls

[g] Adenoma

[h] Bile duct hamartoma

[i] Chromophobe adenoma

[j] Cortical adnoma

[k] Fibroma

[l] Fibroadenoma

[m] Pancreatic lipoma

[n] Follicular cell adenoma incidence significant (p=0.021, Fisher exact test) compared with pooled controls

[o] Stromal polyp

In the case of mice, greater toxicity of toxaphene was reported (NCI, 1979). As in the case of rats, survival in treated groups was high; 90-98% of treated and control animals survived beyond the 52nd week of the study. Of the tumors appearing in treated animals, none were observed in greater incidence compared with controls with the exception of those in the liver. Hepatocellular carcinomas occurred in treated mice only, with incidences of 69% and 98% in males (Table V-10) at the low and high doses, and 10% and 69% in females (Table V-11) at the low and high doses, respectively. These neoplasms were not observed in control animals of either sex, but hepatic nodules were observed in 20% of matched-control males, though not in females. On the basis of these findings the authors concluded that toxaphene caused increases in the incidence of hepatocellular carcinomas and neoplastic nodules, and hence was carcinogenic in B6C3F1 mice.

Reexamination of the histological sections prepared in the above study led Reuber (1979) to come to qualitatively similar, but quantitatively different conclusions. In this regard he reported liver carcinoma incidences of 20, 78 and 100% in male mice ingesting zero, low or high doses of toxaphene and 0, 31 and 89% respective incidences in females. In addition one male mouse ingesting the low concentration developed an angiosarcoma of the liver, and one female an adenocarcinoma of the salivary gland. In addition, Reuber reported incidences of 0, 12 and 31% of stromal cell carcinoma in the uterus of females in control, low-dose and high-dose groups, respectively, the latter incidence being the only statistically significant result. No mention was made of these carcinomas in the original NCI (1979) report.

TABLE V-10

Incidence of Tumors in Male Mice Fed Toxaphene in the Diet[a]

Dose (mg/kg diet)	Number of Animals		Blood	Liver	Total[b]	Lung[c]	Eye	Total[b]
	Initial	Examined						
			MALIGNANT			BENIGN		
0 Matched	10	10	0/10[d]	2/10(20%)	0	1/10(10%)	0/10	1
Low	50	50	2/50(4%)	6/49(12%)[e] 34/49(69%)[f] 1/49(2%)	37	1/49(2%)	1/50(2%)	2
High	50	46	0/50	45/46(98%)[f]	45	2/46(4%)	0/50	2

[a] Source: NCI, 1979

[b] Total = total animals with tumors

[c] Alveolar/bronchiolar adenoma

[d] $\frac{\text{Number of animals bearing tumor stated}}{\text{Number of animals with tissue stated examined microscopically}}$

[e] 12% were described to have "neoplastic nodules," assumed to be malignant, 69% hepatocellular carcinoma and 2% angiosarcoma

[f] Incidence of hepatocellular carcinomas in low and high dose groups significant ($p<0.001$, Fisher exact test) compared with matched or pooled controls.

TABLE V-11

Incidence of Tumors in Female Mice Fed Toxaphene in the Diet[a]

Dose (mg/kg diet)	Number of Animals: Initial	Number of Animals: Examined	Hematopoietic System	Liver	Total[b]	Salivary	Mammary	Uterus	Harderian Gland	Total[b]
			MALIGNANT			BENIGN				
0 Matched	10	10	0/10[c]	0/9	0	0/8	0/10	0/10	0/10	1
Low	50	49	1/50(2%)	5/49(10%)[d] 13/49(27%)[d]	6	1/46(2%)	1/50(2%)	2/48(4%)	0/50	4
High	50	49	0/50	34/49(69%)[e,f] 6/49(12%)[e,f]	34	0/47	0/50	0/44	1/50(2%)	1

[a] Source: NCI, 1979

[b] Total = total animals with tumors

[c] $\frac{\text{Number of animals bearing tumor stated}}{\text{Number of animals with tissue stated examined microscopically}}$

[d] 27% were reported to have "neoplastic nodules," (ascribed malignant) and 10% hepatocellular carcinoma

[e] 12% were reported to have "neoplastic nodules," and 69% hepatocellular carcinoma

[f] Incidence of hepatocellular carcinomas significant (p<0.001, Fisher exact test) compared with matched or pooled control groups

In addition to the previous study, a number of other much less complete studies on toxaphene carcinogenicity were conducted. In a study conducted by the U.S. FDA (Nelson, 1949), groups of 12 male and 12 female rats assumed to be of the Osborne-Mendel strain ingested 0, 25, 100, 400 or 1600 ppm toxaphene in the diet for 107 weeks. Further groups of 5 male and 5 female rats were given 0, 40, 200 or 1000 ppm toxaphene and sacrificed after 56 weeks. Tissues were sectioned only from the first set of groups, however, and incomplete histology was performed on other groups. Only one neoplasm was noted, a subcutaneous neurosarcoma in a male rat fed the 1000 ppm dose level, although liver hyperplasia and hyperplastic nodules were reported. Thyroid hyperplasia was also noted in treated animals, especially in males.

In the groups constituting the 107-week experiment, 2/3 female and 2/2 male rats ingesting 1600 ppm toxaphene developed hepatic carcinomas; 1/3 female rats in each of the 400 and 1600 ppm groups developed hyperplastic nodules. No incidence of either of the above tumors was observed in control or other treatment groups (Nelson, 1949).

A further study demonstrating a statistically-significant increase in hepatic neoplasms was also done (Litton Bionetics, Inc., 1978). Fifty-four weanling B6C3F1 mice were randomly assigned to each of four groups. Toxaphene (X-16189-49), as well as the dose levels, were selected and provided by Hercules Inc. The animals were administered toxaphene at 0, 7, 20 and 50 ppm levels in the diet for 18 months and surviving mice were kept on the control diet for another 6 months. All survivors were killed 24 months after the initiation of treatment. Table V-12 shows the effects of toxaphene on survival. The survival in both treated and control groups was similar.

TABLE V-12

Survival of Mice fed Toxaphene in the Diet[a]

Sex	Dose (ppm)	No. at Start	Weeks -- Died or Killed in a Moribund Condition			
			1-26	27-52	53-78	79-105
Male	0	54	0	0	1	10
	7	54	0	0	0	8
	20	54	0	1	3	6
	50	54	1[b]	0	0	7
Female	0	54	0	0	0	10
	7	54	0	0	2	7
	20	54	1	1	2	8
	50	53	0	0	0	9

[a] Source: Litton Bionetics, Inc., 1978

[b] Missing

The histopathological evaluation data presented in Table V-13 shows a statistically significant incidence of hepatocellular tumors (hepatocellular carcinomas and hepatocellular adenomas) in male mice fed 50 ppm toxaphene in the diet as compared with the controls. However, hepatocellular tumor incidence was not statistically significant in females as compared with controls.

Toxaphene was administered to 50 weanling ARS Golden Syrian hamsters of each sex and each dose at levels of 0, 100, 300 and 1000 ppm based on a subchronic study (Litton Bionetics, Inc., 1979). The hamsters, obtained from the Sprague-Dawley laboratory in Madison, WI, were randomly assigned to each group. The toxaphene was mixed in corn oil and blended with the food that was available ad libitum. The animals were observed during a 15-day acclimation period before initiation of the study and daily afterward for general condition and mortality Treatment was continued for 18 months for the females and 21.5 months for the males who showed a high survival at 18 months.

Animals were observed until spontaneous death, unless moribund, or surviving until termination of the experiment. Moribund animals and those surviving until termination were sacrificed. All animals were necropsied. The postmortem observations included a thorough external examination and collection of major organs and representative tissues from all animals. All collected tissues from control and high-dose animals, and target organs and gross abnormalities from the low- and intermediate-dose animals, were evaluated histopathologically. Blood smears were taken from all animals at sacrifice, but were not examined unless necessary for definition of specific lesions or diseases. A compound-related lowered body weight was observed in

TABLE V-13

Hepatocellular Tumor Incidence in B6C3F1 Mice fed Toxaphene in the Diet*

Dose (ppm)	Hepatocellular Tumor Incidence	
	Males	Females
0	10/53 (19%)	2/53 (4%)
7	10/54 (19%)	2/53 (4%)
20	12/53 (23%)	4/53 (8%)
50	18/51 (35%)	6/52 (12%)

*Source: Litton Bionetics, Inc., 1978

high-dose males until 12 months of study. Similar effects were noted in males receiving 300 ppm from months 2-7 and for a brief period in females receiving 1000 ppm. At terminal sacrifice, an increase in liver weight was observed in males at the 1000 ppm dose, which correlated to the pathologic finding of megahepatocytes in 6 of 21 males observed. A decrease in heart weight was noted in males at both the 300 and 1000 ppm dose levels. A decrease in the thyroid gland weights of the 1000 ppm females was also noted.

Only the liver changes were supported by histopathology and were therefore judged to be treatment-related. These liver changes are similar to those accompanying an induction of liver enzymes after administration of chlorinated hydrocarbons and other compounds. Microscopic evaluation of the tissues did not reveal any incidence of a tumorigenic effect related to toxaphene. Even when the incidence of lymphoreticular neoplasms of all types was combined, no difference was detected between controls and dosed animals. Under conditions of this study, toxaphene was not carcinogenic.

It is likely that the maximum tolerated dose was not employed in these studies (Litton Bionetics, Inc., 1979). In the subchronic study, the maximum tolerated dose estimate was based on evidence of liver toxicity only. No hamster at any dose level died during the study; no changes in general appearance, condition or behavior among experimental animals was observed; and measurements of mean body weights and food consumption indicated no compound-related change. In the chronic study, a dose-related decrease in body weight was observed, but it was temporary. The weight of treated animals equaled or surpassed that of controls by the end of 1 year for all treatment groups. Treated animals also survived for a longer period than controls. A comparison of the number of animals in each treatment group surviving to termination of the experiment using an X^2 test for homogeneity revealed significant differences for both males ($p<0.05$) and females ($p<0.001$).

Intragastric injection of toxaphene in sunflower oil solution in a dose of 50 mg/kg bw twice-weekly for 10 weeks was reported to give no excess tumor incidence in mice (18 g initial weight) (Didenko et al., 1978). The mouse strain was apparently indeterminate (stated as nonlinear), and both sexes were used. Although the sites of the tumors in each sex were not reported, the "tumor formation frequencies" of 12.5% for treated, and 8.9% for control groups were reported to be not significantly different 60 weeks after the start of the experiment. Of toxaphene-treated mice, 8 bore pulmonary adenomas, 1 adenocarcinoma, and 1 lymphogranulomatosis. In the control group, five mice had pulmonary adenomas. In a parallel study in rats, animals of 80 g weight were injected twice-weekly for 16 weeks with 80 mg toxaphene/kg bw in sunflower oil. No primary data were presented but toxaphene injection was reported not to increase the frequency of tumor formation compared with controls, or to induce differences in tumor distribution relative to gender. Both control and treated animals were reported to develop leukemia and lung tumors by the age of 2.

In a study on the effects of toxaphene on spontaneous and BaP-induced carcinogenesis in female A/J mice (Triolo et al., 1982), the pesticide was added to the diet in a fashion such that control diets contained 5% corn oil and experimental diets 5% corn oil and 100 or 200 ppm technical grade toxaphene. Mice were 9 weeks old on initiation of the study, and were maintained on these diets for 12 or 20 weeks. Following the 12-week experiment essentially no tumors of any kind were found in the forestomach, lung, liver, glandular stomach, intestinal tract or spleen from control or experimental groups. When mice were intubated with 3 mg BaP in 0.25 mℓ corn oil on days 7 and 21 by oral intubation, 100 ppm toxaphene in the diet had no effect on BaP-induced forestomach tumorigenesis, but 200 ppm caused a statistically significant decrease in tumor incidence. In the case of the lung, 200 ppm was without effect, but 100 ppm resulted in decreased tumor (presumably adenoma) incidence significantly compared with BaP alone. Concurrently, the same dietary concentrations of toxaphene were found to significantly increase control and BaP-induced hydroxylase activities in the liver, but to reduce them in lung (Triolo et al., 1982).

In the 20-week portion of this experiment BaP-treated mice ingested toxaphene at 100 ppm in the diet; a significant decrease in the number of lung tumors per mouse was observed compared with mice treated with BaP alone. In mice treated with the pesticide alone, the incidence of tumors was low and identical to untreated controls. The incidence of forestomach tumors in the animals receiving BaP and toxaphene was reported to be too large to quantify accurately. Similar effects were seen on BaP-hydroxylase activity after 20 weeks of toxaphene ingestion to those observed after a 12-week feeding period (Triolo et al., 1982).

In order to assess the carcinogenic activity of toxaphene _in vitro_, the effect of the pesticide at concentrations of 0.3-100 μM on C3H10T1/2CL8 cell transformation induced by 4 μM BaP was investigated (Nesnow and Leavitt, 1979). Of a number of agents tested, toxaphene was least active but was reported to increase the BaP induced transformation frequency in a dose-related manner. No primary data were presented.

Summary

Acute oral exposure of mammals and birds to toxaphene resulted in LD_{50} values ranging from 7.5-10 mg/kg bw in female monkeys to 75-500 mg/kg in rabbits (see Table V-1). The LD_{50} values resulting from dermal application of toxaphene are generally higher than those observed after oral exposure and range from ~250 mg/kg bw to ~4000 mg/kg, both values being reported for rabbits (see Table V-2). Toxaphene fractions or components differed in chemical composition, polarity and solubility leading to >10-fold differences in toxicity. The vehicle in which the pesticide is solubilized for administration also appears to have an effect on the toxic response. Specific components of technical toxaphene are more toxic than the complex mixture, and as reported for mice, LD_{50} values for several components varied from 2.5 to >100 mg/kg bw (see Table V-3). Brain levels of toxaphene may be indicative of acute toxicity (see Table V-4). In swine, >4 mg/kg wet weight (brain tissue) constituted a lethal level, and 2 mg/kg was associated with clinical signs of toxicity.

Subchronic oral doses of toxaphene in laboratory animals resulted in few clinical signs of poisoning. Responses varied from lethality in several mice given 1280 ppm in the diet for 6 weeks to no apparent adverse effects in rats following ingestion of feed containing 189 ppm toxaphene (see Table V-5). At doses <189 ppm in the diet, subclinical toxicity was reported such as decreased bile production and questionable liver pathology. In mice receiving 100 or 200 ppm toxaphene in the diet, humoral antibody production (IgG antibody formation) was suppressed; however, cell-mediated immune responses were not affected.

Toxaphene aerosols in the form of dusts are apparently more toxic to rats than toxaphene mists. Mist concentrations as high as 500 mg toxaphene/m^3 for 3 weeks caused no mortality in rats and rabbits; at 12 mg toxaphene dust/m^3 for 3 months, mortality occurred in rats, dogs and guinea pigs. These results may be a function of particle size.

Long-term exposures of animals to dietary levels of toxaphene are summarized in Tables V-6 through V-11. In a number of studies (see Table V-6), no adverse effects were reported among the parameters monitored in each experiment. In several lifetime studies in rats, no effects were reported for doses between 10 and 100 ppm toxaphene in the diet; other studies at 100 ppm reported liver pathology. The lowest dietary level producing liver damage in dogs was 5 ppm in the diet for 2 years. In addition to liver pathology, a variety of adverse effects are reported from both *in vivo* and *in vitro* studies: kidney pathology, decreased bile flow, decreased survival, inhibition of intestinal transport of glucose, inhibition of gluconeogenesis, inhibition of mitochondrial enzymes, inhibition of Na^+/K^+-ATPase brain and kidney activity and Mg^{2+}-ATPase activity inhibited in mouse kidney, liver and brain.

Toxaphene induces the microsomal MFO system, and treatment of an animal *in vivo* with a cytochrome P-450 system inhibitor increases toxaphene toxicity.

In a 3-generation study with 25 or 100 ppm toxaphene in the feed of Sprague-Dawley rats, no effects on parental animals or offspring could be detected. With CD rats, maternal mortality was 31% at 35 mg toxaphene/kg bw and there was a dose related decrease in maternal weight gain and fetal weight (15, 25 or 35 mg/kg bw). No adverse effects were reported in the offspring of mixed-strain white rats given 4 mg/kg bw during the entire pregnancy. In hamsters given the same dose, the authors reported toxaphene to be teratogenic but did not identify the anomaly.

More sensitive endpoints in rats were examined by several investigators. Toxaphene (oral), at 12 mg/kg bw given to pregnant rats, depressed cholinesterase activity in fetal cardiac neural structures and delayed cardiac neural differentiation. At 25 mg/kg bw, of the many biochemical parameters examined, the only significant difference was noted in the kidney (decrease in alkaline phosphatase activity and total protein). There was no effect at 12.5 mg/kg bw on the rat kidney. In two separate experiments, there was little evidence of behavorial deficits in the offspring of toxaphene treated dams except for retarded righting ability (6 mg/kg and 50 µg/kg bw).

Toxaphene, given to pregnant mice (15 mg/kg and 25 mg/kg bw) had no adverse effect on parameters examined in offspring. In this same study the high dose (35 mg/kg bw) induced encephaloceles in five litters of 90 treated dams. A 5-6 generation study with toxaphene (25 ppm) in the diet resulted in little or no adverse effect in mice. No effects were noted on the anatomical development of the fetal guinea pig from pregnant females given 15 mg/kg bw from days 21-35 of gestation.

Toxaphene appears to have a low teratogenic potential unless doses are high enough to induce maternal toxicity. In the studies reported here, the lowest dose producing an effect was 50 μg/kg bw (rats) given in the diet from day 5 of gestation through termination of the study; effects included significant decreases in overall swimming ability and righting ability of young pups when toxaphene was administered before postnatal day 16.

Toxaphene is mutagenic in the Salmonella/microsomal reverse mutation assay using strains TA98 and TA100. Results of assays of toxaphene components for their mutagenic potential indicated that mutagenicity resided in the polar fraction. Mutagenicity was decreased by the addition of active MFOs, S9; this agrees with the *in vivo* observation that inhibitors of cytochrome P-450 increased toxicity. The mutagenic activity of commercial preparations varies.

Studies of toxaphene carcinogenicity are reported in Tables V-8 through V-14. In one study with carcinogenicity as the endpoint (NCI, 1979), many doses were lowered because of overt toxicity in the rats and mice ingesting toxaphene containing feed. The concurrent numbers of control animals were low (10/sex/group) and historical controls were used in order to match the number of treated animals per group (50 of each sex/group). The range of doses given to the animals makes it difficult to use TWAs of doses as a basis on which to estimate risk for humans (Table V-14). It was concluded that, under the conditions of the bioassay, toxaphene is carcinogenic in male and female B6C3F1 mice, causing increased incidences of hepatocellular carcinomas in a dose-related manner. The results also revealed that toxaphene is carcinogenic for the thyroid of male and female Osborne-Mendel rats.

TABLE V-14

Variation in Doses of Toxaphene Used in NCI (1979) Study of Carcinogenicity

Species	Sex	Time-Weighted Average Dose (ppm in feed)	Range of Dose (ppm in feed)
Rat[a]	male	1112	640-2560
		556	320-1280
	female	1080	640-1280
		540	320-640
Mouse[b]	male female	198	160-320
	male female	99	80-160

[a] Increased incidence of thyroid tumors in both males and females at the high doses.

[b] Increased incidence of hepatocellular carcinoma in males and females at both doses.

In another study with B6C3F1 mice of both sexes, using 7, 20 and 50 ppm toxaphene in the diet, the carcinogenicity of toxaphene was demonstrated at these much lower doses. After 18 months of toxaphene ingestion and 6 months on a control diet, there were increased incidences of hepatocellular carcinoma in males. The response in females was less pronounced.

Reports from the U.S. FDA study (Osborne-Mendel rats) included one subcutaneous neurosarcoma in a male rat given 1000 mg toxaphene/kg diet, liver hyperplasia and hyperplastic nodules, and thyroid hyperplasia.

A significant increase in hepatocellular tumors was detected in mice in two separate studies. When adenomas and carcinomas are combined, a statistically significant increase in thyroid tumors was noted in both male and female rats along with a significant increase in the cumulative incidence of pituitary tumors in female rats. These findings are considered sufficient evidence to classify toxaphene as an animal carcinogen. Using the IARC criteria for classifying the carcinogenic evidence with "sufficient" animal data, toxaphene is ranked in the IARC 2B group, meaning that toxaphene is probably carcinogenic in humans. Classification according to the EPA guidelines for carcinogens classifies toxaphene in group B2: Probable Human Carcinogen, meaning that there is sufficient evidence of carcinogenicity from animal studies and inadequate or no data from epidemiologic studies.

VI. HEALTH EFFECTS IN HUMANS

Acute Toxicity

Toxaphene, like most insecticides, is poisonous by swallowing, skin contact, or inhalation (Boots Hercules Agrochemicals, Inc., n.d.). The following properties are cited in Boots Hercules Agrochemicals, Inc., undated. Emulsions or solutions in vegetable oils facilitate skin or gastrointestinal absorption. Solutions in kerosene are hazardous, but absorption is much more "erratic." When toxaphene is in the form of a powder, dermal absorption is negligible, but inhalation exposure of respirable particles may be considerable. Toxaphene poisoning in humans is manifested by diffuse stimulation of the central nervous system resulting in salivation, restlessness, hyperexcitability, muscle tremors or spasms, generalized convulsions, and sometimes loss of consciousness. Nausea and vomiting may follow oral ingestion. Clonic convulsions may also occur and can be prevented by barbiturates (i.e. sodium pentobarbital) administered preferably by i.v. Toxaphene has a fairly long duration of action (i.e. over several hours) and a long-acting barbiturate such as phenobarbital can be used after initial control of convulsions. In lethal cases, convulsions continue until death, which is ultimately caused by respiratory failure.

The IUPAC (1979) has estimated an acute oral LD_{50} dose of 60 mg toxaphene/kg bw. Hayes (1975) reported a level of 78 ppm in the liver of a dead person poisoned with toxaphene as well as 10 ppm in blood. Conley (1952) estimated an acute lethal dose to be between 2 and 7 g/person or 29-100 mg/kg for a 70 kg man.

Fatal intoxications of humans have been caused by "cotton dust" containing 5% DDT, 10% toxaphene and 40% sulfur. The "subacute fatal human dose" for this mixture is reported to be 0.75 g DDT + 1.5 g toxaphene (Heyndrickx, 1960).

Several cases of death from toxaphene poisoning have been recorded (Hayes, 1963). A 9-month-old child poisoned with a 2:1 mixture of toxaphene:DDT died after convulsions and respiratory arrest. Toxaphene was detected in the liver (7.9 ppm), kidney (6.8 ppm) and brain (14 ppm). The ratio of toxaphene:DDT in the brain and liver was 10:1, and in the kidneys, 3:1 (Haun and Cueto, 1967). Four other cases of acute poisoning in children, 3 of them fatal, have been reported (McGee et al., 1952). Table VI-1 summarizes the findings of this study. No residue data were reported. Recovery of humans from poisoning occurred at doses of 10-47 mg/kg bw. Vomiting was induced so it is not clear how much toxaphene was absorbed.

Poisoning by prolonged skin contact with a lindane/toxaphene solution has been reported by Pollock (1958). The patient was semiconscious, disoriented and stuporous, nauseous and had vomited many times. Severe epigastric pain, severe aches, and pains of the extremities were also reported. Eventually stiff neck, dehydration, and irregular pulse developed. Treatment was with penicillin, chloromycetin and digitalis. The atypical symptoms may be related to the presence of lindane or the dermal portal of entry. No residue data were given. Hayes (1963) estimated a hazardous dermal dose to be 46 g. For a 70 kg man, this is equivalent to 660 mg/kg or about 10 times that of an oral dose.

TABLE VI-1

Case Studies of Toxaphene Poisoning in Humans in which Ingestion Was the Primary Route of Entry*

Case No.	1	2	3	4	5	6
Subject(s)	Male, 2 years 8 months	Male, 4 years	Male, 1 year 5 months	Male, 2 years	Female, 49 years Female, 20 years Female, 16 years Female, 12 years	Male, adult Male, young
Nature of toxaphene	Wax	Emulsion in water	60% in solvents	20% in solution	Residue of spray in food	Residue of spray in food
Dose	Unknown	Unknown	Unknown	Unknown	9.5-47 mg/kg	Unknown
Time to react symptoms	7 hours	2 hours	NS	NS	1.5-4 hours	4 hours
Symptoms	Convulsions	Convulsions 2-5 minute intervals	Convulsions intermittent	Convulsions, intermittent mild cerebral excitement; aimless jerking motion and excessive muscular tensions of extremities, marked pharyngeal and laryngeal spasms; labored respiration; cyanosis	Nausea; vomiting convulsions	No nausea; spontaneous vomiting; convulsions, jerking and transitory movements; muscular rigidity; periods of unconsciousness; amnesia(?)
Outcome	Death	Death	Death	Recovery	Recovery	Recovery
Time of death or recovery	9.5 hours	6 hours	11 hours	12 hours	12 hours	24 hours

*Source: McGee et al., 1952

NS - Not specified

Allergic bronchopneumonia was immediately observed in two workers who had just used toxaphene sprays (Warraki, 1963). One had been exposed to other pesticides for 4 years and showed marked bilateral hilar lymphadenopathy with fine miliary opacities heavily distributed over both lungs which, after treatment with dihydrostreptomycin and isoniazid, regressed. The other man also showed similar symptoms, and no past pesticide exposure was evident apart from that to toxaphene. The miliary shadows regressed when the man was treated with streptomycin, isoniazid, prednisone and cortisone. Both cases evidenced the following: sudden exertional dyspnea, tachycardia and weakness with low blood pressure; extensive miliary shadows in both lungs; blood eosinophilia; high serum globulins; reversibility of X-ray shadows, symptoms and physical signs to normal on treatment with cortisone under cover of antituberculosis drugs; and acute pneumonia symptoms.

Eight women working in an area that had been sprayed with 2 kg toxaphene/ha by aircraft showed a higher incidence of chromosome aberrations (acentric fragments and chromatid exchanges), as observed in lymphocyte cultures, compared with an unspecified number of control individuals: 13.1% vs. 1.6% (Samosh, 1974).

The sequelae observed after a pesticide fire in which ~80 fire fighters were potentially heavily exposed to toxaphene amongst many pesticides (Melius and Schulte, 1981) were generally vague (tension, depression, hyperexcitability, fatigue, and confusion were significant relative to the 24 fire fighters who reported acute symptoms of nausea, dizziness, headache at the time of the fire).

Subchronic and Chronic Toxicity

Application of an aerosol spray containing toxaphene to the skin of 50 human subjects daily for 30 days at a dose of 300 mg/day produced no toxic manifestations. Fifty human volunteers who inhaled 0.4 mg/m³ of toxaphene aerosol for 10 minutes a day for 15 days had no subjective or objective effects. Assuming alveolar ventilation of 5.4 ℓ/minute the daily dose equalled ~0.02 mg. A mist containing 250 mg/m³ toxaphene was inhaled by 25 humans for 30 minutes each day (~41 mg/day) for 13 days and they showed no evidence of local or systemic toxic manifestations (Shelanski, 1947).

Many studies exist that involve long-term toxaphene exposure to workers. Most of them do not detail exposure data, involve many other pesticides, and do not attempt to correlate serum levels of toxaphene or its metabolites to known symptoms of toxaphene so that the results, while suggestive, are very often not definitive. Examples of studies of this kind follow. One was a health study detailing an increased incidence of lung cancer (10 observed vs. 0.54 expected) among 285 pesticide applicators (Barthel, 1976). Another was a survey involving 321 exposed and 46 nonexposed males in Kuwait in which the 260 exposed to organochlorines reported increased sputum and hyperreflexia compared with controls (El-Desouky et al., 1978). A third study was a nonsignificant case-control study of the association between the yearly rate of fatal aplastic anemia and the use of many pesticides in the USA between 1950 and 1975 (Wang and Grufferman, 1981); a fourth investigation was a cancer morbidity study (Barthel, 1981) in 1658 male workers of at least 5 years experience as agricultural plant protection workers or plant protection agronomists in the German Democratic Republic between 1948 and 1972. Toxaphene exposures occurred after 1954. An incidence of 169 malignant neoplasms was found. The ones with a significant excess compared with the general population were bronchial carcinoma, 59 (35%) to 42 (24%), respectively, of which 22 were undifferentiated and small-cell carcinomas, 14 were squamous epithelium carcinomas, 3 were adenocarcinomas, 2 were solid carcinomas and one was an alveolar cell carcinoma. The average latency period for the lung cancers was calculated to be 17±6 years (6-29 years) and these cases had been exposed on the average for 14±5 years (6-23 years). Smoking was not a significant factor in these cases. Toxaphene was thought not to be a significant factor in these incidences.

In a survey of 199 employees who worked or had worked with toxaphene between 1949 and 1977, with exposures ranging from 6 months to 26 years (mean 5.23 years), 20 employees died, one with cancer of the colon. None of these deaths could be attributed to toxaphene (IARC, 1979).

No increased porphyrin production was found in the urine of 45 workers exposed daily to a variety of pesticides, predominantly parathion, toxaphene, DDT and dieldrin. The qualitative method used was capable of detecting 0.4 μg porphyrins/mℓ. No correlations were found between serum pesticide levels and urinary excretion of ALA, PBG and CCA. Parathion depressed plasma or red cell ChE or both in five workers. These findings suggest that ordinary occupational exposure to the pesticides noted above has no strong porphyrinogenic or sympathotonic effects (Embry et al., 1972).

Summary

Toxaphene has caused convulsions and nausea in humans when exposure has occurred by swallowing, skin contact or inhalation. The acute oral LD_{50} has been estimated in one study to be 29-100 mg/kg, and 60 mg toxaphene/kg bw in another. Mixtures of toxaphene with DDT (2:1, by weight) are more acutely toxic than either component alone, the subacute fatal human doses being 0.75 g DDT and 1.5 g toxaphene. The liver, brain and kidney can show residues. The estimated hazardous dermal dose is ~660 mg/kg bw for liquid toxaphene. Allergic bronchopneumonia has been observed after acute inhalation of toxaphene sprays.

Dermal doses of 300 mg/day for 30 days, and inhaled doses of 0.4 mg/m³ for 10 minutes on each of 15 days, and 250 mg/m³ for 30 minutes (~41 mg/day) on each of 13 days caused no atypical subjective or clinical effects.

Studies in the workplace are confounded because exposure to many chemicals occurred in all the reported studies. There are no porphyrinogenic or sympathotonic effects mentioned in the available literature.

Human data are inadequate to assess the human carcinogenic potential for toxaphene.

VII. MECHANISMS OF TOXICITY

Introduction

Toxaphene toxicity is influenced by many factors: age, gender, species, state of microsomal-enzyme systems, presence of starvation or of other chemicals that may affect cytochrome P-450 levels, and the type of carrier utilized in toxicity experiments (see Chapter V).

In all cases the obvious acute effects in humans and animals (salivation, hyperexcitability, behavioral changes, muscle spasms, convulsions) indicate that the central nervous system is the chief target system. The mechanism of the effect is still unknown. Other target organs affected acutely include the kidneys (cloudy swelling, congestion, renal tubular degeneration), the liver (fatty degeneration, necrosis, inhibition of bile flow and biliary function), the testes (decreased spermatogenesis) and the skeleton (altered cartilaginous structures in birds and in guinea pig fetuses).

At least in Swiss-Webster albino mice (Saleh et al., 1977; Saleh and Casida, 1979), enhanced acute toxicity (oral LD_{50} values) was associated with introduction of a single chloro-group in the 8- or 9-position and to a lesser extent in the 5-exo-position. A decrease in toxicity for heptachlorobornanes was noted for single chloro-groups in the 3-exo or 10-positions. For the nonachlorobornanes, additional chloro-groups in the 3-exo, 10-positions or in the 8, 10-positions caused compounds to be much less acutely toxic. The hexachlorobornane produced by removal of a chlorine at the 6-position from the heptachlorobornane was not much more toxic than the heptachlorobornane itself.

Enzymatic Effects

Khalifa et al. (1976) and Chandurkar and Matsumura (1979a) found that toxaphene and Toxicants A and B could be reductively dechlorinated or dehydrochlorinated quickly or their vicinal chloride eliminated by liver microsomal preparations in vitro as well as nonenzymically by reduced hematin (Khalifa et al., 1976). Clearly, certain toxaphene components will be metabolized faster than others. In vitro metabolism of toxaphene by isolated microsomal preparations from mice required NADPH and relatively anaerobic conditions (Khalifa et al., 1976). The fact that toxaphene gave type I binding spectra with the hepatic cytochrome P-450 of rats, mice, sheep and rabbits is suggestive that toxaphene components are substrates for the hepatic microsomal mixed-function oxidase system (Kulkarni et al., 1975). This is also supported by the following: the toxicity of toxaphene can be potentiated by piperonyl butoxide (Turner et al., 1977); toxaphene causes shortened pentobarbital sleeping times in rats (Schwabe and Wendling, 1967; Trottman and Desaiah, 1980); shortened N-methyl cyclohexenylymethyl barbiturate sleeping times in rats have been measured (Ghazal, 1965); transient decreases in (Peakall, 1976) and estrone (Welch et al., 1971) metabolism; enhanced microsomal enzyme activity (Peakall, 1976; Trottman and Desaiah, 1980; Pollock et al., 1983); increases for pentobarbital hydroxylation (Trottman and Desaiah, 1980), aminopyrine demethylase and aldrin epoxidation (Pollock et al., 1983) and the level of cytochrome P-450 (Pollock et al., 1983) and NADPH cytochrome c-reductase (Trottman and Desaiah, 1980) being dose-dependent. The "no significant hepatoinductive effect" level for i.p. administered toxaphene is between 5 and 25 mg/kg bw for these enzymes (Pollock et al., 1983).

Saleh and Casida (1978) showed that the major products of Toxicant B produced by rat liver microsomes under anaerobic conditions were CAS RN 57981-29-0 and 65620-64-6 in a 2:1 ratio; these products were not produced under aerobic or anaerobic conditions if NADPH was absent. These products were found directly in fat, liver and feces of adult male Sprague-Dawley rats as well as CAS RN 64618-63-9 after a gavage dose of 3.1 mg Toxicant B/kg bw. Chandurkar and Matsumura (1979a) reported that in addition to a pathway requiring NADPH, there appeared to be a pathway utilizing glutathione, as found by EC/GC product analysis and inhibitor experiments using ^{36}Cl- and ^{14}C-toxaphene, and Toxicants B and C as substrates. In addition, the NADPH pathway metabolized Toxicant C much more rapidly than Toxicant B. Glucuronides, sulfonates and acid hydrolysable conjugates were also found. The addition of UDP-glucuronic acid to toxaphene/NADPH system increased the extent of metabolism by 8-fold, but not when Sesamex (an inhibitor of NADPH) was also present, also showing probable involvement of the glucuronic acid metabolic pathway. The water soluble metabolites found when glutathione (GSH) was added were thought to be GSHconjugates formed involving loss of a chloride. Chandurkar and Matsumura (1979a) also identified the major dechlorination product of Toxicant C to be CAS RN 70459-31-3, and 4 secondary alcohols (hydroxyls at C_2, C_3, C_5, C_6 positions) and one primary alcohol. Oxidation products of Toxicant B were not alcohols. Thus, different components in toxaphene, not surprisingly, are metabolized differently. Ionic-chlorine is the major excretion product in both feces and urine (Crowder and Dindal, 1974; Ohsawa et al., 1975), and small amounts of urine and fecal metabolites are glucuronides or sulfate conjugates (Chandurkar, 1977). The small amounts of conjugates may support the observation by Mehendale (1978) that toxaphene inhibits hepatobiliary function, at least in rats.

Mehrle et al. (1979) proposed that the skeletal effects in birds could be explained by a functional deficiency of vitamin C in the backbone resulting from shunting vitamin C to the liver to enable the organism to detoxify toxaphene in the liver by mixed function oxidase activity, as is also proposed for the "broken back syndrome" caused by toxaphene for fish (IUPAC, 1979). Birds capable of synthesizing vitamin C in the liver (e.g., ravens, sparrows) should not be so susceptible as those that cannot (e.g. ducks, geese, quail, turkeys, doves, pigeons). This has not yet been shown experimentally however.

DiPasquale (1977) showed that 15 mg toxaphene/kg bw/day (day 21 to day 35 of gestation) increased collagen-containing structures in guinea pig fetuses, and this was thought to be due to a functional deficiency of vitamin C related to maternal mixed-function oxidase induction.

How the vitamin C regulatory shunt interacts with the hepatic NADPH and glutathione systems in the metabolism of toxaphene is still unknown, and it is unclear whether there is a link between events in the liver and those induced in the central nervous system.

Deichmann and Keplinger (1970) noted that pretreatment of rats with aldrin or dieldrin raised 96-hour LD_{50} values 2-fold, and 3-fold after pretreatment with p,p-DDT. Since these compounds are all known to induce hepatic mixed function oxidase activities, this may mean that compounds that induce this system will lower the acute toxicity of toxaphene. This was also so when toxaphene was administered together with parathion, diazinon or trithion with which toxaphene is often co-formulated (Keplinger and Deichmann, 1967). The potentiation of toxaphene toxicity with piperonyl butoxide (Turner et al., 1977; Saleh and Casida, 1978) and perhaps the effect of starvation (Boyd and Taylor, 1971; Srebocan et al., 1980a) may be mediated through inhibition of the activity of microsomal enzymes although this has not been shown experimentally.

Subacute administration of toxaphene also appears to affect enzymatic regulation of carbohydrate metabolism in young poultry (Srebocan et al., 1978, 1980b). Thus, pyruvate:CO_2 lipase (ADP) and GTP:oxaloacetate carboxylase (transphosphorylating) in cockerel livers were significantly decreased by doses >5 mg/kg diet after 2 weeks of exposure. The authors evoked adrenocortical mediation as the mechanism. The activities of beef heart mitochondrial succinoxidase and NADH-oxidase were inhibited _in vitro_ by 330 μM toxaphene (Pardini et al., 1971). Guthrie et al. (1974) reported that at 100 μM, the active transport of glucose through isolated mouse intestine was inhibited. The _in vitro_ inhibition of brain, kidney and liver Na^+/K^+-ATPases (IC_{50}, 30 μM) and oligomycinsensitive (IC_{50}, 15 μM) and insensitive Mg^{2+}-ATPase activities from male mice also implicates an inhibition of membrane transport systems (Trottman and Desaiah, 1979, 1983; Fattah and Crowder, 1980). Subacute blood chemistry phenomena include: increased serum acid phosphatase, glutamic pyruvic transaminase, gamma-glutamyl transpeptidase activities, and increased neutrophil counts for dosed mice (Bäumler, 1975). This behavior is consistent with mild liver pathology.

The major enzymic effect caused by chronic exposure appears to be unbalanced sugar metabolism. At a dose to rats of 35 mg toxaphene/kg bw/day for 6 months (Gertig and Nowaczyk, 1975), depressed (17-20%) LDH activities occurred in the liver, kidney and serum, as well as decreased (50%) serum alkaline phosphatase and liver glutamate dehydrogenase. Peakall (1979) showed that levels of pyruvic and lactic acids in blood plasma were not affected even after 6 months at a dose of 2.4 mg toxaphene/kg bw/day and postulated that the effects on the LDH system came from "rats stressed in some way." However, Desaiah et al. (1979) found dosedependent increases in liver microsomal glucose-6-phosphatase and fructose1,6-diphosphatase after 8 weeks of dosing Sprague-Dawley rats with doses of 0, 25, 50 and 75 mg toxaphene/kg diet. This was indicative of changes in the process of gluconeogenesis. Alekhina and Kuz'minskaya (1980) found that LDH activities in myocardium increased after 4 months of dosing "white rats" with 2.7 mg toxaphene/kg bw/day, and in the liver only after both oral and percutaneous exposure (9.4 mg/kg bw/day). Ishikawa et al. (1978) reported that a single i.p. treatments of 40 mg toxaphene/kg bw/day caused decreased plasma cholesterol at day 60 with no effects on plasma triglycerides, liver weights, microsomal protein or cytochrome P-450 in old Sprague-Dawley rats. No dose-related effects were observed on brain Na^+/K^+-ATPases or oligomycin-sensitive and -insensitive Mg^{2+}-ATPases for doses of 0, 50, 100, 150 and 200 mg toxaphene/kg chow for 8 weeks to adult Sprague-Dawley rats, a 30-40% decrease being observed for doses above 50 mg/kg diet. Since _in vitro_ tests showed dose-effect relationships, it was postulated that _in vivo_ toxaphene metabolized before it reached the brain. This was evident also for those enzyme activities in the brain of male ICR rats dosed at 0, 10 25 and 50 mg/kg bw/day over 3 days, though the activities of kidney ATPases except for the oligomycin-sensitive Mg^{2+}-ATPase were decreased in a dose-dependent manner as was liver— oligomycin-sensitive Mg^{2+}-ATPase (Trottman and Desaiah, 1979). Parvu et al. (1980) observed that 4 mg toxaphene/kg bw/day for 30 days to Wistar rats caused lipid accumulation in the liver, depletion of free fatty acids, and increased liver cholesterol.

These data reveal that the major target organs for chronic toxicity effects are, in order of decreasing importance: liver (probably by imbalances in sugar metabolism and ATPase activity depression), kidney (probably by kidney ATPases), heart (catecholamine metabolism distortion; probable ATPase effects), the central nervous system (catecholamine metabolism distortion; generalized tremors in chronically dosed Osborne-Mendel rats), and the immune system (premature aging effects in mice and rats). In birds, the skeleton is probably as important a target organ as the liver (though this may also occur in young mammals) because of the inability of some birds to synthesize vitamin C in the liver necessary for detoxifying the administered toxaphene.

Mutagenic Activity

Toxaphene, and specifically a polar subfraction, is a direct-acting mutagen. It is not clear from the literature what the identities are of the compounds in the polar subfraction, or whether, like most of the nonpolar components, they are metabolized so quickly that in fact metabolites are the direct-acting mutagens. Clarification of this mechanism is an important research need. Toxaphene has been found to be carcinogenic in two animal species, Osborne-Mendel rats and $B6C3F_1$ mice in studies described in Tables V-8 to V-11 (NCI, 1979). The molecular events and the role of the polar fraction have not been formulated. While rats tend to show malignancies of the endocrine system (the thyroid and the pituitary in females) mice showed cancers only in the liver. This does suggest the link between the liver and central nervous system could be by effects on the endocrine system.

Summary

Figure VII-1 summarizes the mechanisms underlying the acute to chronic effects of toxaphene.

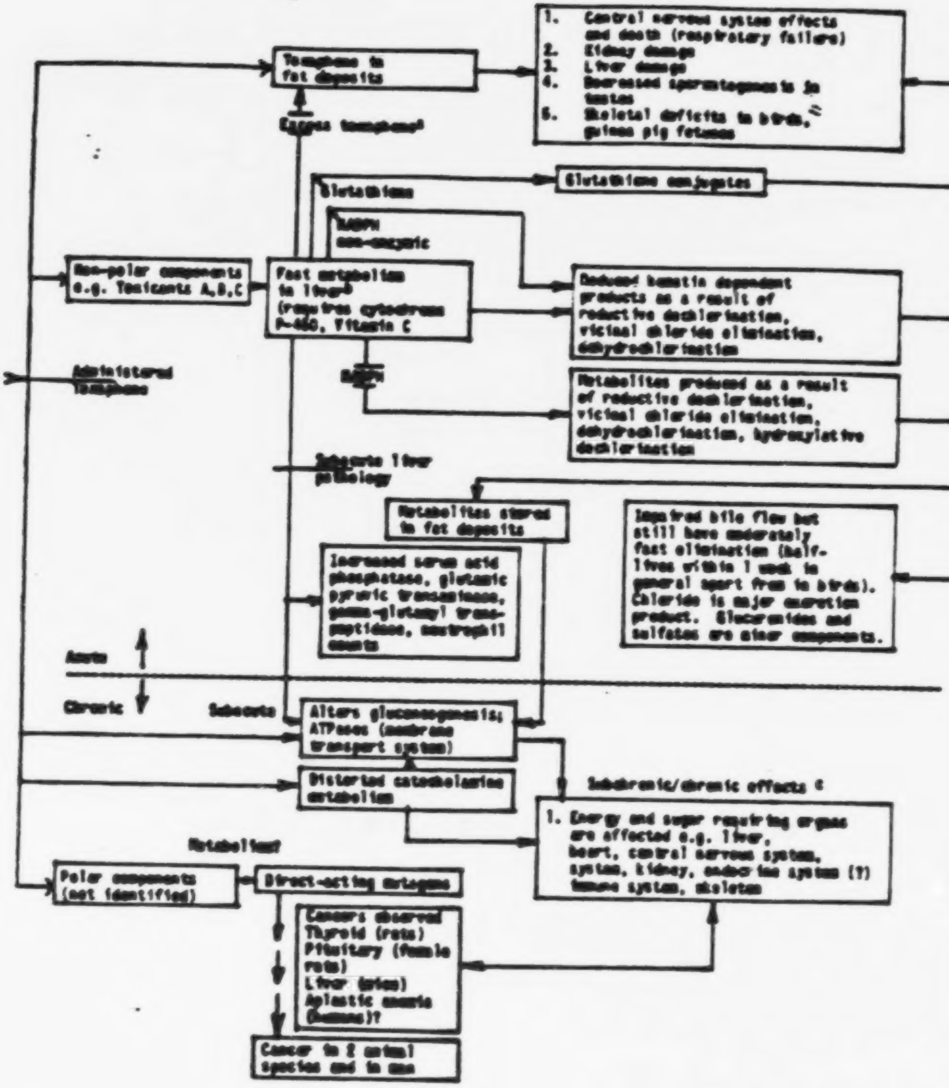
Toxaphene in fat deposits
Excess toxaphene[a]
1. Central nervous system effects and death (respiratory failure)
2. Kidney damage
3. Liver damage
4. Decreased spermatogenesis in testes
5. Skeletal deficits in birds, guinea pig fetuses
Glutathione
Glutathione conjugates
NADPH non-enzymic
Non-polar components e.g. Toxicants A,B,C
Fast metabolism in liver[b] (requires cytochrome P-450, Vitamin C
Reduced hematin dependent products as a result of reductive dechlorination, vicinal chloride elimination, dehydrochlorination
NADPH
Administered Toxaphene
Metabolites produced as a result of reductive dechlorination, vicinal chloride elimination, dehydrochlorination, hydroxylative dechlorination
Subacute liver pathology
Metabolites stored in fat deposits
Increased serum acid phosphatase, glutamic pyruvic transaminase, gamma-glutamyl transpeptidase, neutrophil counts
Impaired bile flow but still have moderately fast elimination (half-lives within 1 week in general apart from in birds). Chloride is major excretion product. Glucuronides and sulfates are minor components.
Acute
Chronic
Subacute
Alters gluconeogenesis; ATPases (membrane transport system)
Distorted catecholamine metabolism
Subchronic/chronic effects[c]
1. Energy and sugar requiring organs are affected e.g. liver, heart, central nervous system, system, kidney, endocrine system (?) immune system, skeleton
Metabolism?
Polar components (not identified)
Direct-acting mutagens
Cancers observed Thyroid (rats) Pituitary (female rats) Liver (mice) Aplastic anemia (humans)?
Cancer in 2 animal species and in man

FIGURE VII-1

Summary of Mechanisms Underlying Acute to Chronic Effects of Toxaphene

[a] 10 mg/kg diet for sheep, steers, cows; 4.74 mg/female ring-necked pheasant/day; 12.5 mg/kg bw/day for female White Leghorns; 21 mg/kg diet for rats, 2.4 mg/kg bw/day causes residue steady state in liver after 1 month and 3 months for brain

[b] No significant inductive effect on affected liver enzymes is between 5 and 25 mg/kg bw for rats.

[c] 1-3 mg/kg bw/day

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Introduction

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Do (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{[Uncertainty Factor(s) x Modifying Factor]}} = \underline{\quad\quad} \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicological effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the U.S. EPA (1986a) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]
- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]
- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]
- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{(\text{RfD}) \times (\text{Body weight in kg})}{\text{Drinking Water Volume in } \ell\text{/day}} = \underline{\quad\quad} \text{ mg/}\ell$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 ℓ/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$\text{HA} = \frac{(\text{NOAEL or LOAEL}) \times (\text{bw})}{(\text{UF}) \times (\underline{\quad\quad}\ \ell\text{/day})} = \underline{\quad\quad} \text{ mg/}\ell$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 ℓ water per day.
2. 10-day HA for a 10 kg child ingesting 1 ℓ water per day.
3. Longer-term HA for a 10 kg child ingesting 1 ℓ water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 ℓ water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

> Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.
>
> Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
>
> Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
>
> Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.
>
> Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually come from lifetime exposure studies using animal. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 ℓ of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Noncarcinogenic Effects

The effects of acute exposure to toxaphene, in humans as in animals, include salivation, hyperexcitability, behavioral changes, muscle spasms, and convulsions, indicating that the CNS is the critical target organ system. In contrast, the liver and the immune system appear to be the critical target sites in chronic or subchronic exposure. Central nervous system effects characteristic of acute intoxication appeared during chronic exposure only at dietary concentrations greatly in excess of those causing liver damage in rats (Kennedy et al., 1973).

Studies of the effects of long-term exposure of workers to toxaphene have been reported. These are of little utility for standard development, however, because exposure generally was poorly defined and often was to a mixture of pesticides, and because no effort was made to correlate effects with plasma or tissue levels of toxaphene or its metabolites.

A few controlled subacute studies of human toxaphene exposure have been reported. None of these resulted in any observed toxicity. Application of an aerosol spray containing toxaphene to the skin of 50 human subjects daily for 30 days at a dose of 300 mg/day produced no toxic manifestations. Fifty human volunteers who inhaled 0.0004 mg/ℓ of toxaphene aerosol for 10 minutes/day for 15 days had no subjective or objective effects. Twenty-five humans inhaling a mist containing 0.25 mg toxaphene/ℓ of air for 30 minutes each day for 13 days showed no local or systemic toxic manifestations (Shelanski, 1947).

Most acute studies to date have involved attempts to define the LD_{50} for a variety of species. Lackey (1949a) also determined the threshold for initiation of convulsions in dogs. Single doses of toxaphene were administered in corn oil by stomach tube at doses varying from 5-50 mg/kg bw in 5 mg/kg increments. No convulsions or mortality occurred in three dogs dosed with 5 mg/kg. Convulsions developed in 4/5 exposed to 10 mg/kg. Mortality was first detected at the 15 mg/kg dose (2/8) with 6/8 developing convulsions. The LD_{50} was estimated to be ~25 mg/kg bw. If repeated doses of toxaphene at 5 mg/kg bw were given, convulsions would develop regularly, but only after several days. Four dogs dosed daily with 4 mg/kg bw, 2 dogs for 44 days and 2 for 106 days, survived although convulsions occurred occasionally. In the subchronic exposures, hydropic degeneration was seen in the liver with degenerative changes in the tubular epithelium of the kidneys.

Olson et al. (1980) reported effects at much lower doses. Behavioral effects were noted in offspring of rats given daily doses of 50 μg/kg from day 5 of gestation until 70-90 days postpartum. Since the effects were generally transient and appeared to result from temporary delays in maturation, it is uncertain whether they can be classified as adverse. Secondly, since the offspring were exposed in utero, by nursing and by toxaphene added to the feed, it is uncertain what actual exposure levels were. Studies such as those of Shaw (1947) indicate that toxaphene may be concentrated in the milk.

There are a number of subchronic and chronic studies of toxaphene toxicity in animals. Many of these document changes in activities of various enzymes, such as lactate dehydrogenase isozymes, serum alkaline phosphatase, and tissue ATPases (Gertig and Nowaczyk, 1975; Desaiah et al., 1979; Trottman and Desaiah, 1979; Alekhina and Kuz'minskaya, 1980). Although some of these observations are suggestive of an effect on gluconeogenesis, they are of doubtful significance since there is no direct proof that glucose metabolism is actually altered, at least in rats (Peakall, 1979).

There is some evidence that suggests that lipid metabolism may be affected by toxaphene exposure. Ishikawa et al. (1978) reported that a single i.p. treatment of old Sprague-Dawley rats with 40 mg toxaphene/kg bw/day resulted in a decrease in plasma cholesterol 60 days later with no effect on plasma triglycerides or on liver weight. On the other hand, Parvu et al. (1980) observed that administration of 4 mg/toxaphene/kg bw/day to Wistar rats for 30 days caused lipid accumulation in the liver, depletion of free fatty acids, and an increase in liver cholesterol.

Recently, Allen et al. (1983) concluded that the immune system is at least as sensitive to the ingestion of toxaphene as the liver. Toxaphene was added to the diet of female Swiss Webster mice at concentrations of 10, 100 and 200 ppm for 8 weeks. Among groups of 23-26 animals each, liver weights were significantly ($p<0.05$) increased in the 100 and 200 ppm groups.

Among groups of 10 animals each, IgG antibody titers were also significantly ($p<0.05$) decreased at the two higher doses. Cell-mediated immune responses, however, were not affected. Offspring of females fed toxaphene in the diet from 3 weeks before mating until weaning were also tested at 8 weeks of age. The cell-mediated immune response was suppressed in the offspring of the 100 ppm group, while the phagocytic ability of macrophages was significantly reduced even in the 10 ppm group. The actual toxaphene dose cannot be estimated accurately in the latter study since the offspring received toxaphene transplacentally, in the milk and possibly even in the feed. It does suggest, however, that neonates may be at greater risk than adults.

In most studies the critical target organ in rats and dogs chronically exposed to toxaphene was the liver. At dietary concentrations of 100 ppm toxaphene in the diet and above, all studies document some form of liver pathology in exposures lasting from 2 years (in rats and dogs) to lifetime (in rats). Toxaphene at 25 ppm in the diet fed to rats over their lifetimes caused an increase in liver weight with minimal liver cell enlargement (Fitzhugh and Nelson, 1951). Over shorter periods of time (up to 12 weeks), dietary toxaphene levels of up to 189 ppm had no measurable effects on male or female albino rats (Clapp et al., 1971).

The NCI (1979) conducted a chronic study with Osborne-Mendel rats and B6C3F1 mice to determine the possible carcinogenicity of toxaphene added to the diet. Each treatment group consisted of 50 rats or mice of each gender, and 10 untreated animals of each gender were matched controls with data from 40 or 45 untreated animals from similar bioassays pooled for statistical evaluations.

Groups of 50 mice of each gender were administered toxaphene for 80 weeks and observed until sacrifice at 90-91 weeks (NCI, 1979). Low-dose males and females received 160 ppm in the diet for 19 weeks followed by 80 ppm for 61 weeks; high-dose males and females received 320 ppm for 19 weeks followed by 160 ppm for 61 weeks. The TWA doses were 99 or 198 ppm for both males and females. Mean body weights of high-dose male mice were lower than those of matched controls but weights of other dose groups were essentially unaffected by toxaphene. Several animals died before week 19 when doses were lowered. Following this, the dosed mice were generally comparable with controls in appearance and behavior during the first year of the study. During the second year, abdominal distention was observed in all dosed groups, but predominantly in the high-dose males. Other clinical signs included alopecia, diarrhea, rough hair coats, and dyspnea. From weeks 60-76 the low-dose males appeared hyperexcitable. After week 75 there were dose-related differences in survival.

Groups of 50 rats of each gender were administered toxaphene for 80 weeks (NCI, 1979) and then observed until survivors were sacrificed at 108-110 weeks (see Table V-7). Low-dose males were given 1280 ppm in the diet for 2 weeks, 640 ppm for 53 weeks and 320 ppm for 25 weeks for a TWA of 556 ppm. High-dose males received 2560 ppm in the diet for 2 weeks, 1280 ppm for 53 weeks and 640 ppm for 25 weeks for a TWA of 1112 ppm. Low-dose females received 640 ppm in the diet for 55 weeks and 320 ppm for 25 weeks for a TWA of 540 ppm. High-dose females received 1280 ppm for 55 weeks and 640 ppm for 25 weeks for a TWA of 1080 ppm.

Mean body weights of the low- and high-dose female rats were lower than those of the matched controls throughout most of the study, whereas weights of low- and high-dose males were essentially unaffected. During the first 16 weeks, dosed males were generally comparable with controls in appearance and behavior, with the exception of high-dose males that appeared hyperactive during week 2 when the doses for male rats were lowered. At week 53, the concentration of toxaphene in the diet was reduced because a majority of the high-dose males and females developed generalized body tremors. Dose-related decreases in survival rates were not observed. Clinical signs usually associated with aging were observed earlier in dosed rats than in controls: alopecia, diarrhea, dyspnea, pale mucous membranes, rough hair coats, dermatitis, ataxia, leg paralysis, epistaxis, hematuria, abdominal distention and vaginal bleeding. Two females, one high-dose and one low-dose, had impaired equilibrium.

In summary, chronic feeding studies suggest that there are no marked species differences in sensitivity among rats, mice and dogs, although mice may be slightly more sensitive than rats to frank effects of toxaphene. On chronic exposure, a dietary level of about 25 mg/kg diet appears to be a NOAEL, and a dietary level of 40 mg/kg diet to be a LOAEL, with liver pathology being the critical toxicity endpoint. Chronic dietary levels above 100 mg toxaphene/kg diet are likely to be associated with frank effects.

Quantification of Noncarcinogenic Effects

Derivation of 1-Day HA. While kidney and liver pathology as well as immune effects are the critical endpoints for chronic exposure to toxaphene, the nervous system is generally more sensitive to acute exposure. The available data suggest a rather sharp threshold for nervous system effects. Lackey (1949a) reported convulsions in dogs exposed daily to toxaphene at 5 mg/kg/day after several days, while 4 mg/kg/day induced few convulsions even with much longer exposures. No convulsions were induced in dogs after a single dose of 5 mg/kg bw, while 10 mg/kg induced convulsions in 4/5 animals. The Lackey (1949a) study is the only one available that accurately defines a NOAEL for very short-term exposures. Dogs appear to be more sensitive to the acute toxic effects of toxaphene than other laboratory species (see Table V-1) with reported LD_{50}s as low as 20-25 mg/kg bw. There is little evidence that humans are more sensitive to toxaphene than dogs. IUPAC (1979) has estimated an oral LD_{50} dose in humans of 60 mg/kg bw, while Conley (1952) estimated the acute lethal dose in humans to range from 29-100 mg/kg. Finally, the nervous system is the critical endpoint for acute toxicity of toxaphene. There is no evidence from the Lackey (1949a) study or from the variety of teratology studies conducted that very short-term exposures at the threshold level for convulsions result in kidney or liver pathology. Using the NOAEL from the Lackey (1949a) study, the 1-day HA is derived as follows:

$$\text{1 day HA child} = \frac{5\ \text{mg/kg} \times 10\ \text{kg}}{100 \times 1\ \ell} = 0.5\ \text{mg}/\ell$$

where:

5 mg/kg = NOAEL for a single oral exposure to dogs (Lackey, 1949a)

10 kg = weight of protected individual (child)

100 = uncertainty factor appropriate for use with a NOAEL from animal data and to protect sensitive members of the human population

1 ℓ/day = assumed volume of water consumed daily by a 10 kg child

It is recommended that 0.5 mg/ℓ for children be accepted as the 1-day HA.

Derivation of 10-Day HA. The majority of subacute exposures have been conducted to evaluate the reproductive or teratologic effects of toxaphene. Chernoff and Carver (1976) reported maternal toxicity of rats and mice at all doses used (15-35 mg/kg) during exposure from days 7-16 of gestation. Kavlock et al. (1982) found increased total protein in fetuses from dams exposed to 12.5 and 25 mg/kg/bw on days 7-16 of gestation in rats. Daily doses of 12 mg/kg in rats on days 1-2 and 6-14 of gestation resulted in smaller litters and decreased cholinesterase activity along myocardial vessels and cells of the atrioventricular node (Badaeva, 1979, 1981). Toxaphene administered by intubation in doses of 4 mg/kg/bw to rats and hamsters on days 7-11 or 1-15 of gestation had no effect upon development, fetal weight, ratios of males to females, the number of fetal deaths or maternal toxicity (Martson and Skepel'skaya 1980b). The NOAEL for maternal toxicity as well as standard reproductive and teratological parameters in laboratory animals appears to be 4 mg/kg bw. Results of the Lackey (1949a) study, however, suggested the NOAEL may be slightly lower in dogs than in rodents. Minimal kidney and liver pathology were reported in dogs exposed to 4 mg/kg bw/day for up to 44 days. It is uncertain if these effects occur with 10 days of exposure. Occasional convulsions were also noted at this dose level.

Using a LOAEL of 4 mg/kg bw/day from the Lackey (1949a) study, the 10-day HA is derived as follows:

$$\text{10-day HA child} = \frac{4\ \text{mg/kg} \times 10\ \text{kg}}{1000 \times 1\ \ell} = 0.04\ \text{mg}/\ell$$

where:

4 mg/kg = LOAEL from oral exposure to dogs (Lackey, 1949a)

10 kg = weight of protected individual (child)

1000 = uncertainty factor appropriate for use with a LOAEL from animal data and to protect sensitive members of the human population

1 ℓ = assumed volume of water consumed daily by a 10 kg child

It is recommended that the 10-day HA of 0.04 mg/ℓ for children derived from the Lackey (1949a) study be accepted.

Derivation of Longer-Term HA. There are no acceptable studies in the available literature for the derivation of a longer-term HA.

Assessment of Lifetime Exposure and Derivation of a DWEL. There are no acceptable studies in the available literature for the derivation of a lifetime DWEL.

Carcinogenic Effects

Although several epidemiology studies have been conducted attempting to correlate cancer incidence with exposure to organochlorine pesticides (Barthel, 1976, 1981; El-Desouky et al., 1978; Wang and Grufferman, 1981), toxaphene was not thought to be a significant factor in the results of any of these studies. In a survey of 199 employees who worked or had worked with toxaphene between 1949 and 1977 with exposures ranging from 6 months to 26 years (mean 5.23 years), 20 employees died, one with cancer of the colon. None of the deaths could be attributed to toxaphene (IARC, 1979). On the basis of the available information, the evidence for carcinogenicity of toxaphene in humans must be classified as inconclusive.

Several investigations of the carcinogenic potential of toxaphene have been carried out in mice and rats. While interpretations of the results of these studies have been subject to challenge, the conclusion is that chronic exposure to toxaphene can induce hepatocellular carcinoma in both mice and rats and shows some evidence of carcinogenic activity at other sites as well.

The most thorough of these studies were performed by Tracor Jitco Co. under contract to the NCI (1979). The diets and the adjustments made in toxaphene concentrations in the diets have been described above under non-carcinogenic effects. Treatment was discontinued after 80 weeks and the studies were terminated 10-11 weeks later (mice) or 28 weeks later (rats). All animals that died during the studies and all animals surviving at the termination of the studies were submitted for pathologic evaluation. Of the male rats, 90% of the high-dose, 94% of the low-dose and all of the control group lived until at least week 52 of the study. Of the female rats, 96% of the high-dose, 92% of the low-dose and all 10 controls survived beyond the 52nd week. Although none of the tumors observed in treated animals were uncommon for the animal strain used, certain tumors and hyperplastic tissues were present with higher incidence in the treated animals. These included thyroid follicular cell adenomas and carcinomas, and hyperplasias. Thyroid follicular cell adenomas and carcinomas combined were found to be elevated statistically in both male and female high-dose groups compared with either concurrent or historical controls from the same laboratory (see Tables V-8 and V-9). In the female rats there was also a statistically significant increase in the cumulative incidence of tumors of the pituitary (chromophobe carcinomas or adenomas) in the high-dose compared with the control group. The authors concluded that toxaphene administration was associated with an increase in thyroid tumor incidence. However, it should be noted that the adenomas included in these evaluations are not classifiable as neoplasms.

Following an independent examination of these histological preparations, Reuber (1979) also concluded that toxaphene administration was associated with an increase in tumor incidence even greater than that reported by NCI (1979). Since it is uncertain why his interpretation of the tissues differs from the original NCI interpretation, it is difficult to draw conclusions from this study.

In the mice as in the rats, survival in treated groups was high, 90-98% of treated and control animals surviving beyond the 52nd week of the study. Of the tumors appearing in treated animals, none were observed in greater incidence compared with controls with the exception of those in the liver. Hepatocellular carcinomas occurred in treated mice only, with incidences of 98% and 69% in males (see Table V-10) at the high and low doses, and 69% and 10% in females (see Table V-11) at the high and low doses, respectively. These neoplasms were not observed in control animals of either sex, but hepatic nodules were observed in 20% of the matched-control males, though not in females. On the basis of these findings, the authors concluded that toxaphene caused increases in the incidence of hepatocellular carcinomas, and hence, was carcinogenic in B6C3F1 mice. Upon examining the tissues, Reuber (1979) came to a qualitatively similar conclusion, although again he reported a greater tumor incidence than NCI (1979).

In addition to the previous study, a number of other much less complete studies on toxaphene carcinogenicity were conducted. In a study conducted by the FDA (Nelson, 1949), groups of 12 male and 12 female rats assumed to be of the Osborne-Mendel strain were fed 0, 25, 100, 400 or 1600 ppm toxaphene in the diet for 107 weeks. Further, groups of 5 male and 5 female rats were fed 0, 40, 200 or 1000 ppm and then sacrificed after 56 weeks. Tissues were sectioned only from the first set of groups; however, an incomplete histology was performed on other groups. Among the second set of animal groups killed after 56 weeks, only one neoplasm was noted, a subcutaneous neurosarcoma in a male rat given 1000 mg toxaphene/kg, although liver hyperplasia and hyperplastic nodules were reported. Thyroid hyperplasia was also noted in treated animals, especially in males.

In the groups used in the 107-week experiment, 2/3 female and 2/2 male rats ingesting 1600 mg toxaphene/kg developed hepatic carcinomas; 1/3 female rats in each of the 400 and 1600 mg/kg dose groups developed hyperplastic nodules. No incidence of either of the above tumors was observed in control or other treatment groups.

Another study in mice demonstrated a statistically significant increase in hepatic neoplasms (Litton Bionetics, Inc., 1978). Fifty-four weanling B6C3F1 mice were randomly assigned to each of four groups. Toxaphene (X-16189-49), as well as the dose levels, were selected and provided by

Hercules Inc. The animals were administered toxaphene at 0, 7, 20 and 50 ppm levels in the diet for 18 months and surviving mice were kept on the control diet for another 6 months. All survivors were killed 24 months after the initiation of treatment. Table V-12 shows the effects of toxaphene on survival. The survival in both treated and control groups was similar.

The histopathological evaluation data presented in Table V-13 shows a statistically significant incidence of hepatocellular tumors (hepatocellular carcinomas and hepatocellular adenomas) in male mice fed 50 ppm toxaphene in the diet as compared with the controls. However, hepatocellular tumor incidence was not statistically significant in females as compared with controls.

Taken together, the results of these studies (Litton Bionetics, 1978; NCI, 1979) demonstrate that toxaphene, when administered chronically to mice at moderate- to high-dose rates, induces hepatocellular carcinomas in Osborne-Mendel rats and in B6C3F1 mice, and may induce tumors at other sites as well.

In another Litton Bionetics (1979) study, toxaphene was administered to 50 weanling ARS Golden Syrian hamsters of each sex and each dose at levels of 0, 100, 300 and 1000 ppm based on a subchronic study. The hamsters, obtained from the Sprague-Dawley laboratory in Madison, WI, were randomly assigned to each group. The toxaphene was mixed in corn oil and blended with the food that was available ad libitum. The animals were observed during a 15-day acclimation period before initiation of the study and daily afterward for general condition and mortality Treatment was continued for 18 months for the females and 21.5 months for the males who showed a high survival at 18 months.

Animals were observed until spontaneous death, unless moribund, or surviving until termination of the experiment. Moribund animals and those surviving until termination were sacrificed. All animals were necropsied. The postmortem observations included a thorough external examination and collection of major organs and representative tissues from all animals. All collected tissues from control and high-dose animals, and target organs and gross abnormalities from the low- and intermediate-dose animals, were evaluated histopathologically. Blood smears were taken from all animals at sacrifice, but were not examined unless necessary for definition of specific lesions or diseases. A compound-related lowered body weight was observed in high-dose males until 12 months of study. Similar effects were noted in males receiving 300 ppm from months 2-7 and for a brief period in females receiving 1000 ppm. At terminal sacrifice, an increase in liver weight was observed in males at the 1000 ppm dose, which correlated to the pathologic finding of megahepatocytes in 6 of 21 males observed. A decrease in heart weight was noted in males at both the 300 and 1000 ppm dose levels. A decrease in the thyroid gland weights of the 1000 ppm females was also noted.

Only the liver changes were supported by histopathology and were therefore judged to be treatment-related. These liver changes are similar to those accompanying an induction of liver enzymes after administration of chlorinated hydrocarbons and other compounds. Microscopic evaluation of the tissues did not reveal any incidence of a tumorigenic effect related to toxaphene. Even when the incidence of lymphoreticular neoplasms of all types was combined, no difference was detected between controls and dosed animals. Under conditions of this study, toxaphene was not carcinogenic.

It is likely that the maximum tolerated dose was not employed in these studies (Litton Bionetics, Inc., 1979). In the subchronic study, the maximum tolerated dose estimate was based on evidence of liver toxicity only. No hamster at any dose level died during the study; no changes in general appearance, condition or behavior among experimental animals was observed; and measurements of mean body weights and food consumption indicated no compound-related change. In the chronic study, a dose-related decrease in body weight was observed, but it was temporary. The weight of treated animals equaled or surpassed that of controls by the end of 1 year for all treatment groups. Treated animals also survived for a longer period than controls. A comparison of the number of animals in each treatment group surviving to termination of the experiment using an X^2 test for homogeneity revealed significant differences for both males ($p<0.05$) and females ($p<0.001$).

Quantification of Carcinogenic Effects

Since the results of two bioassays (NCI, 1979; Litton Bionetics, Inc., 1978) were positive for cancer induction, estimated risk levels for toxaphene in drinking water can be calculated using a linearized multistage model as discussed in the appendices to the October 1980 Federal Register notice regarding the availability of Water Quality Criteria Documents (Federal Register, 1980). Both studies are considered suitable for the derivation of a criterion. The Litton Bionetics study was selected because it allowed derivation of a slightly more conservative criterion. The animal data used in the derivation are as follows:

Dose (mg/kg/day)	Incidence of Hepatocellular Carcinoma in Male Mice (No. Responding/No. Tested)
0.0	10/53
0.91	10/54
2.6	12/53
6.5	18/51

Length of exposure = 18 months
Length of study = 24 months
Duration of Lifetime = 24 months
Body weight = 0.030 kg

With these parameters, the carcinogenic potency factor for humans q_1^* is 1.131 $(mg/kg/day)^{-1}$. The upper-limit unit risk estimates from the animal data are derived from a linearized multistage nonthreshold extrapolation model which is currently programmed as GLOBAL 83. Justification for its use is presented in EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986b). While recognizing that alternative statistical modeling approaches exist [e.g., One-hit, Weibull, Log-Probit, and Logit models, and maximum likelihood estimates], the range of risks described by using any of these modelling approaches has little biological significance unless data can be used to support the selection of one model over another. In the interest of approach consistency and of providing an upper bound estimate for the potential cancer risk, the Agency recommends the use of the linearized multistage model. EPA considers this model and resulting risk estimate to be an upper limit value in the sense that the true risk is unlikely to be higher and may be lower even zero. An established procedure does not yet exist for making "most likely" or "best" estimates of risk within the range of uncertainty derived by the upper and lower limit values. In order to derive a drinking water concentration of toxaphene calculated to keep individual risk below 10^{-5}, but not taking into account ingestion of seafood or intake of toxaphene by other sources or other routes, the following equation can be used.

$$\text{Water Concentration} = \frac{10^{-5} \times 70\ \text{kg}}{1.131\ \text{mg/kg} \times 2\ \ell} = 0.31\ \mu\text{g}/\ell$$

where:

10^{-5} = risk Level
70 kg = body weight of adult human
1.131 mg/kg = carcinogenic potency factor (human $q_1{}^*$)
2 ℓ = daily water intake for an average adult

For risk levels of 10^{-4}, 10^{-5} and 10^{-6}, the corresponding exposure levels are 3.1, 0.31 and 0.031 µg/ℓ, respectively.

In summary, toxaphene has been shown to induce cancer in two species of animals, while evidence for cancer induction in humans is inconclusive. Toxaphene is, therefore, classified as an animal carcinogen, IARC Category 2B ranking, meaning that toxaphene is probably carcinogenic in humans. Applying the criteria described in EPA's guidelines for assessment of carcinogenic risk (U.S. EPA, 1986b), toxaphene may be classified as Group B2: probable human carcinogen, meaning there is inadequate evidence from human studies and sufficient evidence from animal studies.

Existing Guidelines, Recommendations and Standards

Standards for toxaphene in air, water and food have been established or recommended by many groups. However, most of these standards were set before the results of the NCI (1979) or Litton Bionetics (1978) bioassays of toxaphene for carcinogenicity were available. On May 25, 1977, the U.S. EPA issued a notice of rebuttable presumption against registration and continued registration of pesticidal products containing toxaphene (Federal Register, 1977). A further notice was issued in the Federal Register (1982) where registration for use under specified conditions was required after December 31, 1983 for scabies treatment of beef cattle and sheep. Depletion of existing toxaphene stockpiles was allowed until December 31, 1986 under limited conditions only. Its sale and distribution for use as an insecticide in no-till corn and dry and southern peas was also permitted until December 31, 1986 (Federal Register, 1982).

The ACGIH (1977a) established a TWA value of 500 $\mu g/m^3$ for toxaphene in the air of the working environment. The ACGIH (1977b) based this standard on unpublished acute and chronic toxicity studies conducted in the 1950s and on comparisons of the toxicity of toxaphene with DDT and lindane. In addition, this group set a tentative short-term exposure limit for toxaphene of 1.0 mg/m^3 (ACGIH, 1977a).

The national interim primary drinking water standard for toxaphene is 5 $\mu g/\ell$ (U.S. EPA, 1976). This standard is based on the reported organoleptic effects of toxaphene at concentrations >5 $\mu g/\ell$ (Sigworth, 1965). A safe level of 25 $\mu g/\ell$ was also calculated based on minimal or no effects in rats after they were fed toxaphene at a concentration of 10 mg/kg in the diet, which was estimated to give an average daily dose of 1 mg/kg bw (Lehman, 1965). This latter calculation used the following assumptions:

weight of rat = 300 g
daily food consumption of rat = 50 g
weight of average human adult = 70 kg
average daily water intake for man = 2 ℓ
safety factor = 500
dietary intake = trace (assume zero)

From these assumptions, the maximum safe daily dose for humans was estimated to be 3.4 µg/kg/bw (U.S. EPA, 1976). It should be noted, however, that the assumption of 50 g daily food consumption for a 300 g rat is probably excessively high. The FDA standard of 5 µg/ℓ for bottled water (Federal Register, 1979) is identical to the U.S. EPA's standard for drinking water.

The National Ambient Water Quality Criterion for the protection of human health is based upon levels that may result in incremental increases in cancer risk over the lifetime. For risk levels of 10^{-5}, 10^{-6} and 10^{-7}, the corresponding criteria are 7.1, 0.71 and 0.07 µg/ℓ, respectively (U.S. EPA, 1980; Federal Register, 1980). If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 7.3, 0.73 and 0.07 µg/ℓ, respectively. The criterion was derived from the development of hepatocellular carcinomas and neoplastic nodules in B6C3F1 male mice given several doses of toxaphene (Litton Bionetics, 1978).

The NRC (1977) estimated the RfD of toxaphene for man at 1.25 µg/kg. This was based on a study by Fitzhugh and Nelson (1951), in which rats evidenced increased liver weight and hepatic cell enlargement after exposure to toxaphene at 25 ppm in the diet for 2 years. In their estimation NRC (1977) assumed the daily dose in rats during the Fitzhugh and Nelson (1951) study was equivalent to 1.25 mg/kg/bw, and the application of a safety factor of 1000 was appropriate. Then, assuming a human body weight of 70 kg and a daily water consumption of 2 ℓ, NRC (1977) set the suggested NOAEL from water at 8.75 µg/ℓ (assigning 20% of the total RfD to water) or 0.44 µg/ℓ (assigning 1% of the total RfD to water).

The U.S. EPA criterion for protection of freshwater life was determined to be 0.013 µg/ℓ as a 24-hour average, and the concentration should not exceed 1.6 µg/ℓ at any time. For saltwater aquatic life the criterion is 0.019 µg/ℓ as a 24-hour average with a maximum of 0.120 µg/ℓ (Federal Register, 1980; U.S. EPA, 1980).

The International Joint Commission of the United States and Canada (1977) has recommended a water standard of 0.008 µg/ℓ for the protection of aquatic life. This standard is based on the study by Mayer and Mehrle (1976) in which they found that toxaphene at 0.039 µg/ℓ caused a significant increase in mortality and a significant decrease in growth in brook trout fry over a 90-day period. The standard of 0.008 µg/ℓ is obtained by applying a safety factor of 5.

Special Groups at Risk

Sensitive Subpopulation. Children may form a sensitive subpopulation for the toxic effects of toxaphene. Most fatal cases of toxaphene poisoning have involved children (Hayes, 1982). It is possible that this is due to a greater incidence of high level exposure such as ingestion of toxaphene solutions. However, there is also some evidence for increased susceptibility in young animals. Offpsring of rats fed only 50 μg/kg bw/day, from 3 weeks before mating until weaning age, showed delayed behavioral development (Olson et al., 1980).

Inadequate diet may result in increased susceptibility to toxaphene. Central nervous system effects appeared earlier and at lower concentrations in rats fed a protein deficient diet (Boyd and Taylor, 1971). Toxaphene-induced inhibition of enzymes involved in carbohydrate metabolism was greater in fasted than in fed chicks (Srebocan et al., 1980a).

Individuals with a less inducible xenobiotic metabolizing enzyme system may constitute a sensitive subpopulation. The importance of the cytochrome P-450 mediated detoxification mechanism was indicated by the 2- to 8-fold increase in the toxicity of Toxicant B in mice after treatment with the cytochrome P-450 inhibitor piperonyl butoxide (Saleh et al., 1977; Turner et al., 1977).

Since toxaphene is a complex mixture of mostly chlorobornanes, differing solubilities may affect the actual concentration of the more toxic components in drinking water. The overall solubility of toxaphene is quite low.

Brooks (1974) reported a mean of ~3 mg/ℓ, while Paris et al. (1977) reported a solubility of ≈0.5 mg/ℓ at 25°C. Almost all of the toxicity in toxaphene is due to Toxicants A and B (Hayes, 1982). Toxicant A, the larger of these two fractions, is less polar than the entire mixture (Pollock and Kilgore, 1980) indicating its solubilty in water is probably less than toxaphene as a whole. Toxicant B is more polar and thus possibly more soluble, but makes up only ~1% of the total volume. Thus, it is unlikely that differing solubilities will constitute a major factor in the toxicity of toxaphene in water. However, since the polar fractions are mutagenic (Hooper et al., 1979) the more water soluble components may be important if mutagenesis is related to cancer.

Toxaphene has been detected in drinking water; 27/58 samples contained toxaphene with 2 samples at levels >0.050 ppb (U.S. EPA, 1977). Toxaphene in drinking water has not been detected in some environmental surveys (Schafer et al., 1969; Schulze et al., 1973; Mattraw, 1975), although it has been detected (up to 0.41 ppb) in drinking water after toxaphene spraying (Nicholson, 1964). Toxaphene in rain (up to 0.5 ppb) has also been detected (Williams and Bidleman, 1978; Harder et al., 1980; Munson, 1976; Bidleman and Christenson, 1979), and this exposure route may be important where rain water is utilized as drinking water.

Interactions. Induction of hepatic microsomal MFO systems appears to account for mosy of the interactions of toxaphene with other compounds. In rats pretreated with aldrin or dieldrin and evidencing increased liver O-dealkylase and O-demethylase activities, toxaphene 96-hour LD_{50} values were ~2 times higher (indicating decreased toxicity) than those of rats given no pretreatment. Similarly, pretreatment with p,p'-DDT, a known inducer of hepatic microsomal MFO, resulted in a 3-fold increase in the 96-hour LD_{50} of toxaphene in rats (Deichmann and Keplinger, 1970).

When administered by oral intubation to rats, equitoxic combinations (LD_{50}s) of toxaphene with parathion, diazinon or trithion were less toxic than would be expected, based on the assumption of additive toxicity and similar action (Keplinger and Deichmann, 1967).

Toxaphene is commonly formulated with methyl parathion, usually in a 2:1 mixture, respectively, for control of certain insect pests of cotton. Chlordimeform is often added to the first formulation for additional control. Combinations of these pesticides were given to male Swiss mice by gastric intubation; 25 mg toxaphene/kg bw in 0.16 mℓ corn oil; 12.5 mg methyl parathion/kg + 25 mg toxaphene/kg; 12.5 mg methyl parathion/kg + 25 mg toxaphene/kg + 3.25 mg chlordimeform/kg (Crowder and Whitson, 1980). In these excretion-retention studies, mortality occurred only in groups receiving formulations containing methyl parathion all within 3 hours of dosing. It appeared that methyl parathion toxicity was slightly enhanced by chlordimeform and slightly lowered by toxaphene but the differences were not significant.

The possible interaction of methyl parathion and toxaphene was also examined on the behavior of offspring of Sprague-Dawley rats exposed to pesticides on days 7 through 15 of pregnancy (Crowder et al., 1980). Pregnant rats were given 1.0 mg/kg bw methyl parathion or 1.0 mg/kg methyl parathion + 2.0 mg/kg toxaphene by oral gavage in 0.1 mℓ corn oil.

Toxaphene alone was given from day 7 until parturition at 6 mg/kg/day. Rat pups exposed in utero to these doses of the pesticides alone or combined demonstrated few significant changes in learning ability as measured by a simple two-choice maze, motor skills or behavior. The only significant change was in the additional time required (4.3 days) for methyl parathion-toxaphene treated litters to develop the righting reflex.

Triolo et al. (1982) investigated in A/J mice the effects of ingested toxaphene on the incidence of lung tumors induced by oral administration of benzo(a)pyrene (BaP). Groups of 9-week-old mice (7-48/group) were fed a diet containing toxaphene (in corn oil) at 100 ppm for 12 weeks or 200 ppm diet for 20 weeks. Two doses of BaP (3 mg each) were given by the oral route: the first BaP dose on day 7 after the initiation of the toxaphene diet, and the second dose on day 21. Mice were sacrificed after 12 or 20 weeks and prepared for tumor analysis and analysis of BaP hydroxylase activity. Mice that received toxaphene in the diet alone, or toxaphene and BaP, showed an increase in BaP hydroxylase activity in the liver and a decrease in enzyme activity in the lung. Inhibition of lung BaP hydroxylase activity was paralleled by a reduction in BaP-induced lung tumors in mice fed toxaphene.

Over a period of 60 days Bogachuk and Filenko (1978) studied the effects of vertical vibration, toxaphene, and a combination of these physical and chemical factors in 80 immature inbred male rats of the Wistar and August strains. Rats were divided into four groups consisting of 10 rats of each strain; group 1, control; group 2, vertical vibration (frequency 50 hertz, amplitude 1.25 mm, 30 min/day); group 3, 0.01 LD_{50} toxaphene perorally

(LD_{50} not given); group 4, combined action of vibration and toxaphene at the same levels as in groups 2 and 3. Experiments were conducted on every group at the same time each day for 60 days and covered the period of rapid growth of the animals. Decreased body weight, kidney weight, volume, linear dimensions and thickness of the cortex were observed in the three treated groups. The changes were most pronounced in the group exposed to the combination of toxaphene and vibration; the combined effects appeared to be additive.

Mount et al. (1980) reported an outbreak of toxaphene poisoning in 10 of 15 crossbred sows in midgestation. The animals ate feed that was poured on the wet floor of a holding pen treated a month earlier with a commercial spray containing 45% toxaphene and 2% lindane. Within 30 minutes, 10 of the animals developed an acute nervous disorder. Two sows died within 2 hours from the onset of clinical signs. In the others, severity of signs diminished without treatment after 2.5 hours. Toxaphene and lindane levels (mg/kg wet weight) found in the tissue were: cerebrum, 2.0 and 0.02; cerebellum, 4.0 and 0.03; and serum (hemolyzed), 0.3 and 0.002, respectively. Although the levels of lindane in the tissues were below levels associated with clinical intoxication, the presence of lindane may have contributed to the severity of the signs.

Starvation may cause greater mortality to toxaphene than optimal diets. Thus, rats fed a protein-deficient diet showed acute oral LD_{50} values of 80±19 mg/kg bw, whereas for animals fed standard laboratory chow the LD_{50} was 220±33 mg/kg. The LD_{50} of rats fed a high protein diet was 293±31 mg toxaphene/kg bw, indicating a protective effect of dietary protein. CNS effects appeared earlier and at lower concentrations than in control rats (Boyd and Taylor, 1971). Toxaphene (5 or 10 ppm in the diet), administered to chicks after 24 hours without food, caused lower activities of glucose-6-phosphatase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase in 1-day-old Nick chick cockerels than did toxaphene or starvation alone (Srebocan et al., 1980a). Heat stress in poultry did not appear to interact significantly with toxaphene (Srebocan et al., 1980b). Based on the absence of toxaphene-induced changes in plasma levels of pyruvic and lactic acids in unstressed rats, Peakall (1979) implied that rats had to be "stressed in some way" to obtain depressed blood LDH activity as claimed by Kuz'minskaya and Alekhina (1976) and Gertig and Nowaczyk (1975).

The toxicity of Toxicant B to mice is increased by a factor of 2-8 by administration of piperonyl butoxide (Saleh et al., 1977; Turner et al., 1977), indicative of the importance of cytochrome P-450 mediated detoxification mechanisms. This would indicate that any chemical that depressed or enhanced microsomal function might affect the ultimate toxicity to toxaphene. It has been postulated that in young white Leghorn chickens the detoxification systems are not yet fully induced, making younger animals more susceptible than older ones (Bush et al., 1977). Thus, age may also be an interacting variable, a fact also noted during behavioral testing (Olson et al., 1980).

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